

APPROVED	O.G. FIG.	
BY	CLASS	SUBCLASS
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Docket/App No.: 1440.1027-005

Title: Anti-Angiogenic Proteins and Fragments and Methods of Thereof

Inventors: Raghuram Kalluri

FIG. 1A

09/543,371

pET22b(+) forward primer:

5'-CGGGATCCT TCT GTT GAT CAC GGC TTC-3' (SEQ ID NO:3)

pET22b(+) reverse primer:

5'-CCCAAGCTT TGT TCT TCT CAT ACA GAC-3' (SEQ ID NO:4)

pPICZαA forward primer:

5'-TTCGGAATTC TCT GTT GAT CAC GGC TTC-3' (SEQ ID NO:15)

pPICZαA reverse primer:

5'-TGCTCTAGAGG TGT TCT TCT CAT ACA GAC TTG GCA-3' (SEQ ID NO:16)

5	10	15	20	25	30	35	40	45						
<u>tct gtt gat cac ggc ttc</u>	ctt	gtg	acc	agg	cat	agt	caa	aca	ata					
50	55	60	65	70	75	80	85	90						
gat	gac	cca	cag	tgt	cct	tct	ggg	acc	aaa	att	ctt	tac	cac	ggg
95	100	105	110	115	120	125	130	135						
tac	tct	ttg	ctc	tac	gtg	caa	ggc	aat	gaa	cgg	gcc	cat	gga	cag
140	145	150	155	160	165	170	175	180						
gac	ttg	ggc	acg	gcc	ggc	agc	tgc	ctg	cgc	aag	ttc	agc	aca	atg
185	190	195	200	205	210	215	220	225						
ccc	ttc	ctg	ttc	tgc	aat	att	aac	aac	gtg	tgc	aac	ttt	gca	tca
230	235	240	245	250	255	260	265	270						
cga	aat	gac	tac	tgc	tac	tgg	ctg	tcc	acc	cct	gag	ccc	atg	ccc
275	280	285	290	295	300	305	310	315						
atg	tca	atg	gca	ccc	atc	acg	ggg	gaa	aac	ata	aga	cca	ttt	att
320	325	330	335	340	345	350	355	360						
agt	agg	tgt	gct	gtg	tgt	gag	gcg	cct	gcc	atg	gtg	atg	gcc	gtg
365	370	375	380	385	390	395	400	405						
cac	agc	cag	acc	att	cag	atc	cca	ccg	tgc	ccc	agc	ggg	tgg	tcc
410	415	420	425	430	435	440	445	450						
tgc	ctg	tgg	atc	ggc	tac	tct	ttt	gtg	atg	cac	acc	agc	gct	ggt
455	460	465	470	475	480	485	490	495						
gca	gaa	ggc	tct	ggc	caa	gcc	ctg	gcg	tcc	ccc	ggc	tcc	tgc	ctg
500	505	510	515	520	525	530	535	540						
gag	gag	ttt	aga	agt	gcg	cca	ttc	atc	gag	tgt	cac	ggc	cgt	ggg
545	550	555	560	565	570	575	580	585						
acc	tgc	aat	tac	tac	gca	aac	gct	tac	agc	ttt	tgg	ctc	gcc	acc
590	595	600	605	610	615	620	625	630						
ata	gag	agg	agc	gag	atg	ttc	aag	aag	cct	acg	ccg	tcc	acc	ttg
635	640	645	650	655	660	665	670	675						
aag	gca	ggg	gag	ctg	cgc	acg	cac	gtc	agc	cgc	<u>tgc</u>	<u>caa</u>	<u>gtc</u>	<u>tgt</u>
680	685	690												
<u>atg</u>	<u>aga</u>	<u>aga</u>	<u>aca</u>	taa										

(SEQ ID NO:1)

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FIG. 1B

5	10	15	20	25	30	35	40	45						
SVD	HGF	LVT	RHS	QTI	DDP	QCP	SGT	KIL	YHG	YSL	LYV	QGN	ERA	HGQ
50	55	60	65	70	75	80	85	90						
DLG	TAG	SCL	RKF	STM	PFL	FCN	INN	VCN	FAS	RND	YSY	WLS	TPE	PMP
95	100	105	110	115	120	125	130	135						
MSM	API	TGE	NIR	PFI	SRC	AVC	EAP	AMV	MAV	HSQ	TIQ	IPP	CPS	GWS
140	145	150	155	160	165	170	175	180						
SLW	IGY	SFV	MHT	SAG	AEG	SGQ	ALA	SPG	SCL	EEF	RSA	PFI	ECH	GRG
185	190	195	200	205	210	215	220	225						
TCN	YYA	NAY	SFW	LAT	IER	SEM	FKK	PTP	STL	KAG	ELR	THV	SRC	QVC

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MRR T

(SEQ ID NO:2)

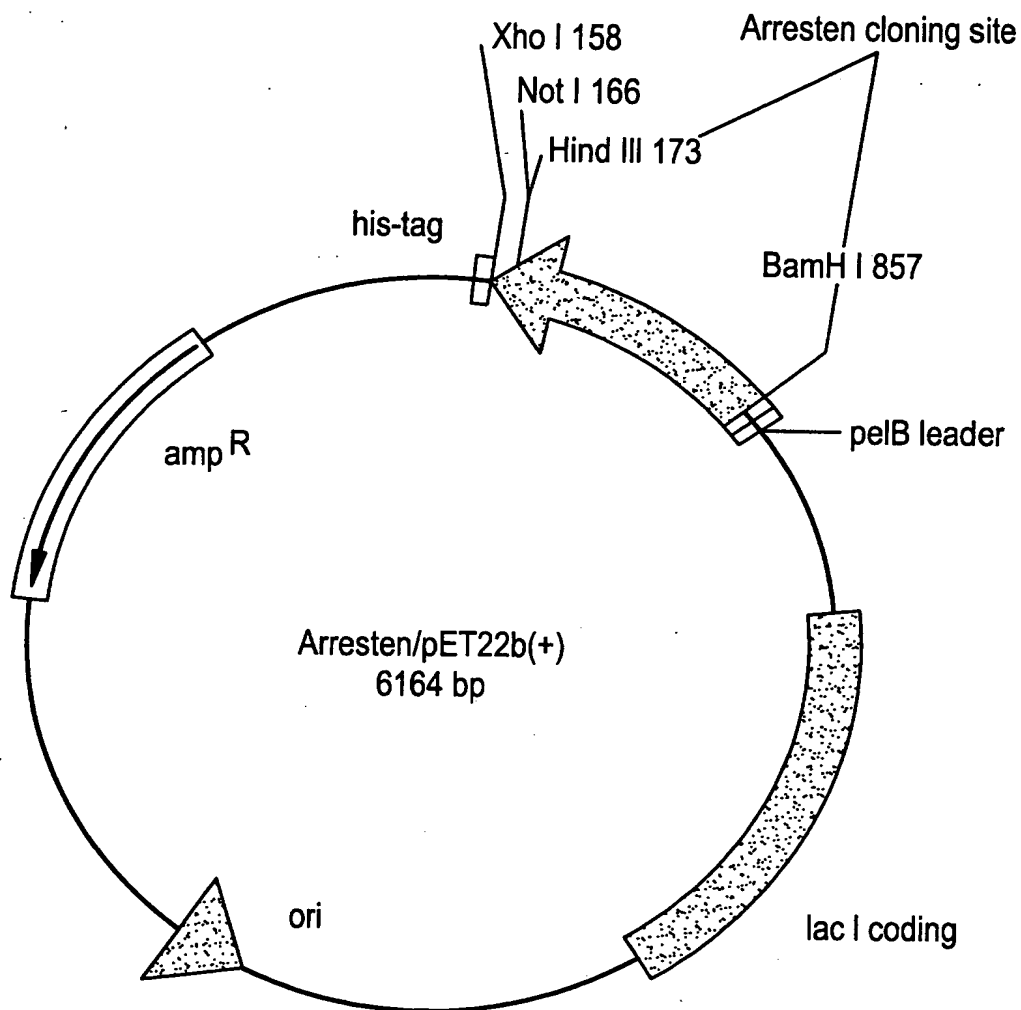
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FIG. 2



Forward primer: 5'-cgggatccttctgttgatcacggcttc-3'

Reverse primer: 5'-cccaagctttgttcttctcatcacagac-3'

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Inventors: Raghuram Kalluri

FIG. 3A

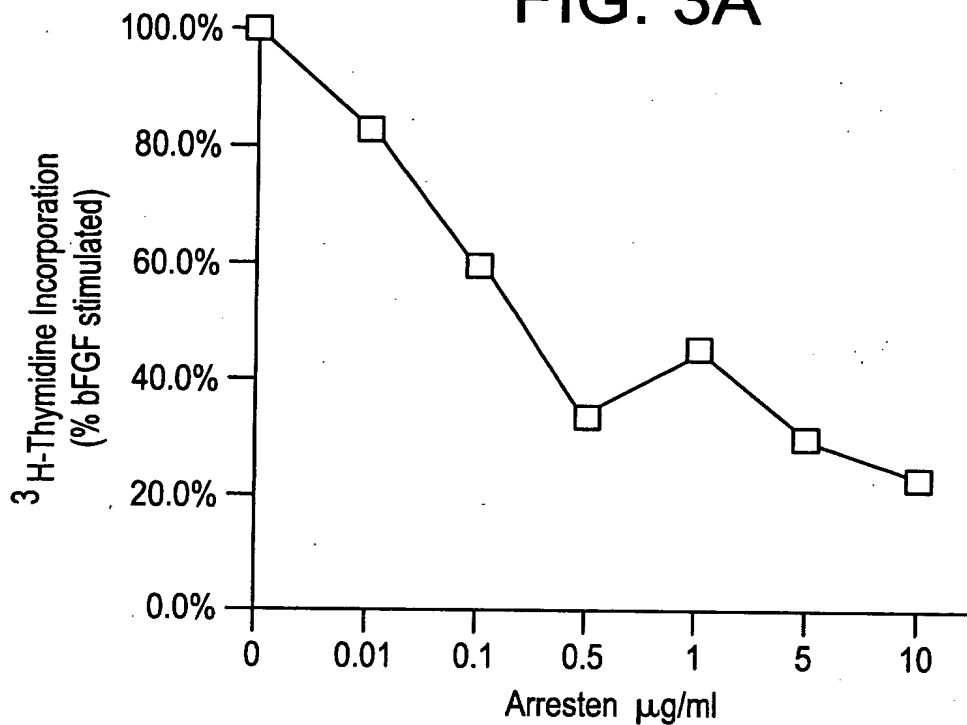
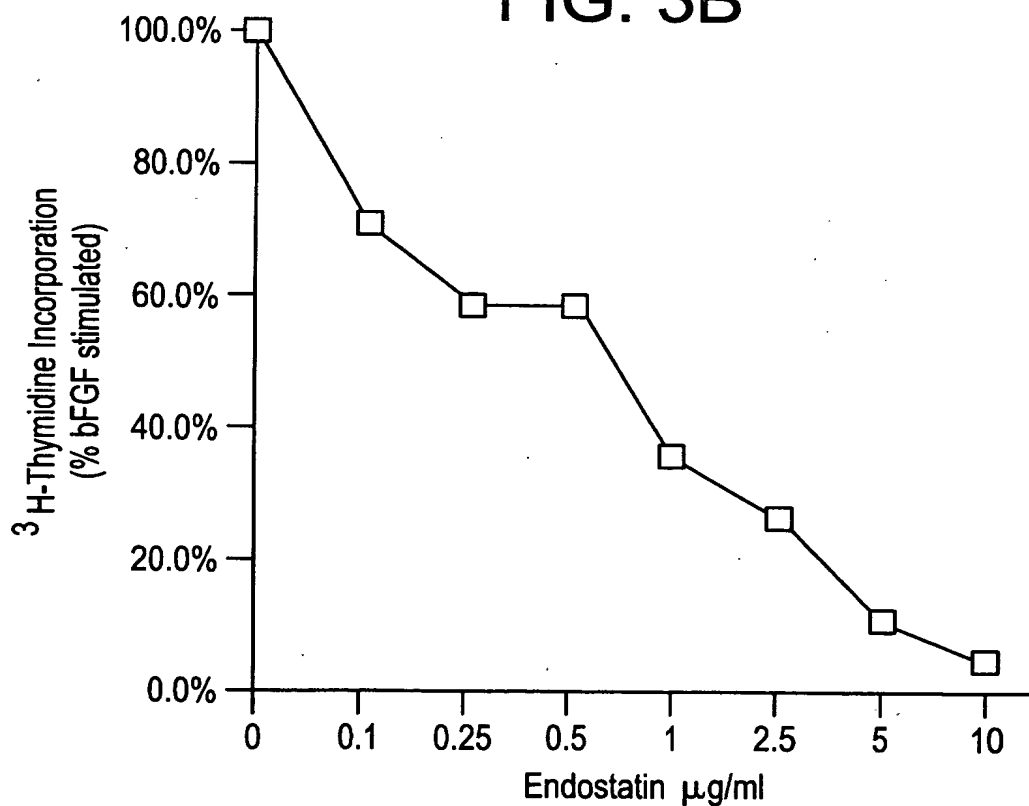


FIG. 3B



APPROVED	O.G. FIG.	
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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

FIG. 4A

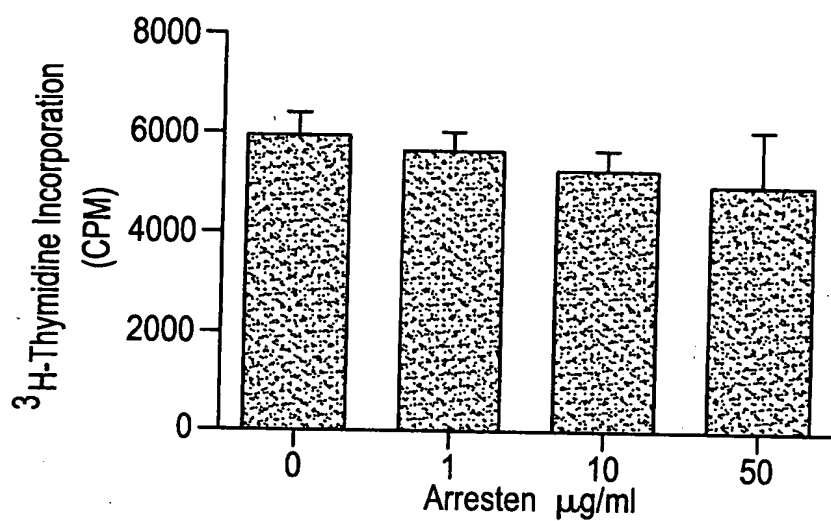
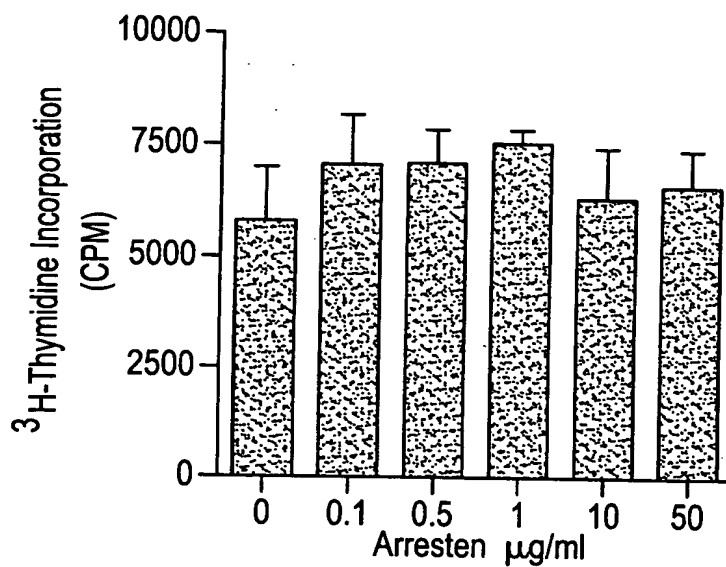


FIG. 4B



APPROVED	O.G. FIG.	
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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

FIG. 4C

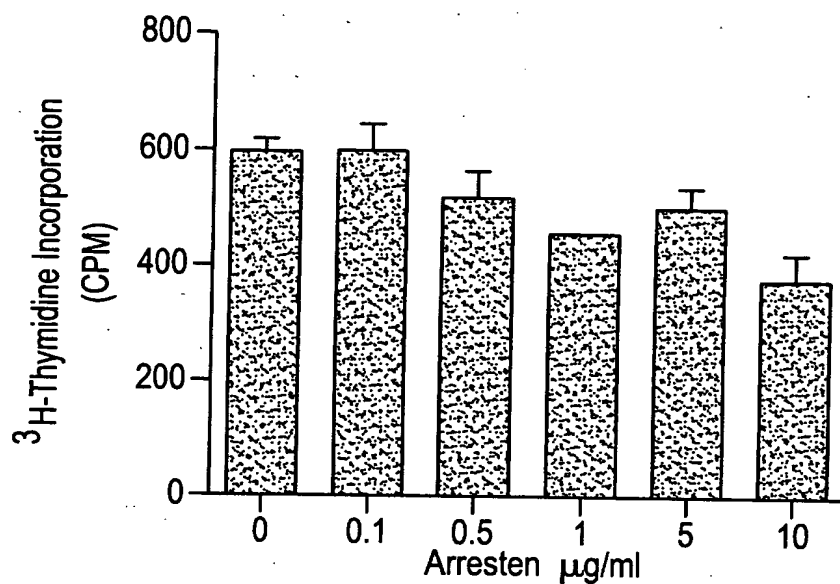
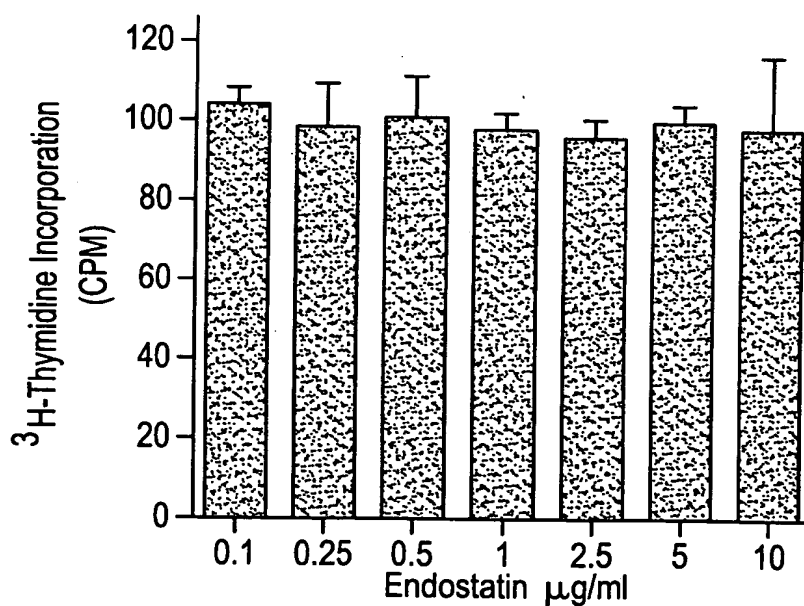


FIG. 4D



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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

FIG. 5A

Control

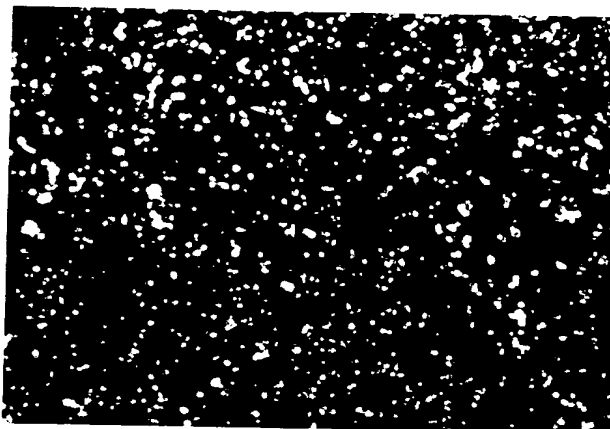


FIG. 5B

Arresten 2 μ g/ml

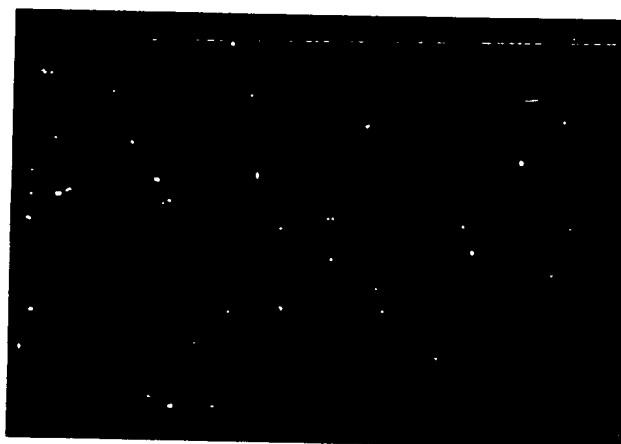


FIG. 5C

Endostatin 20 μ g/ml

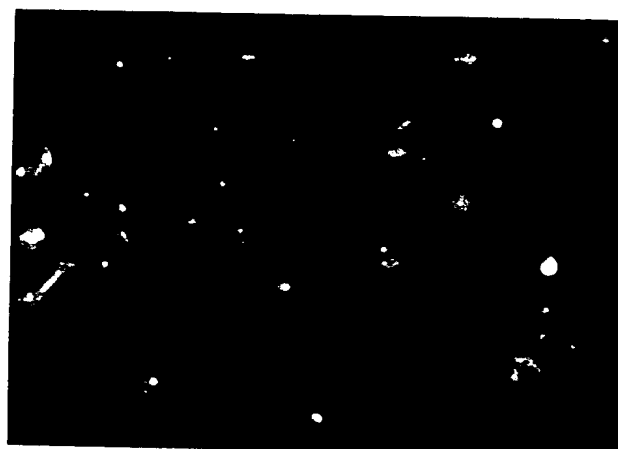


FIG. 6

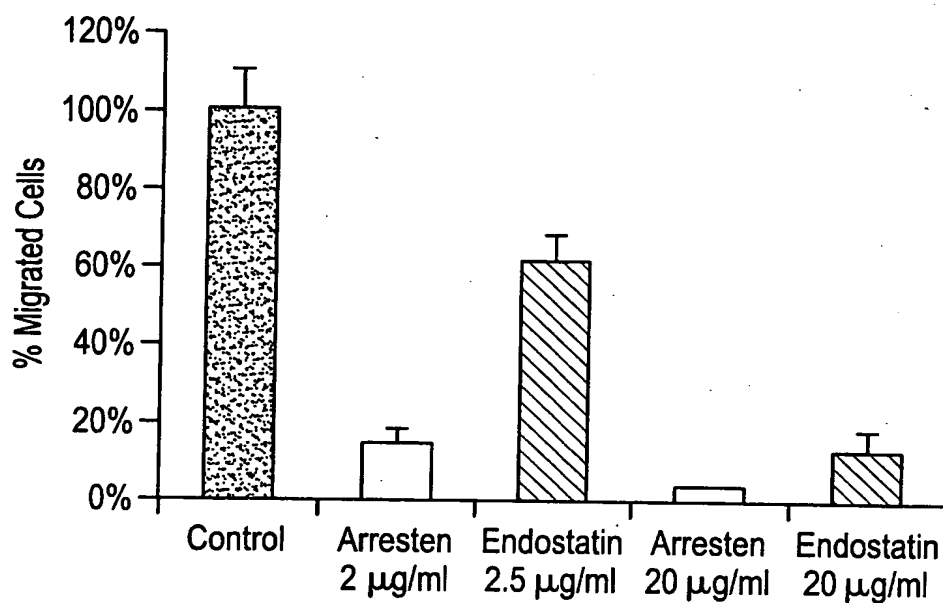
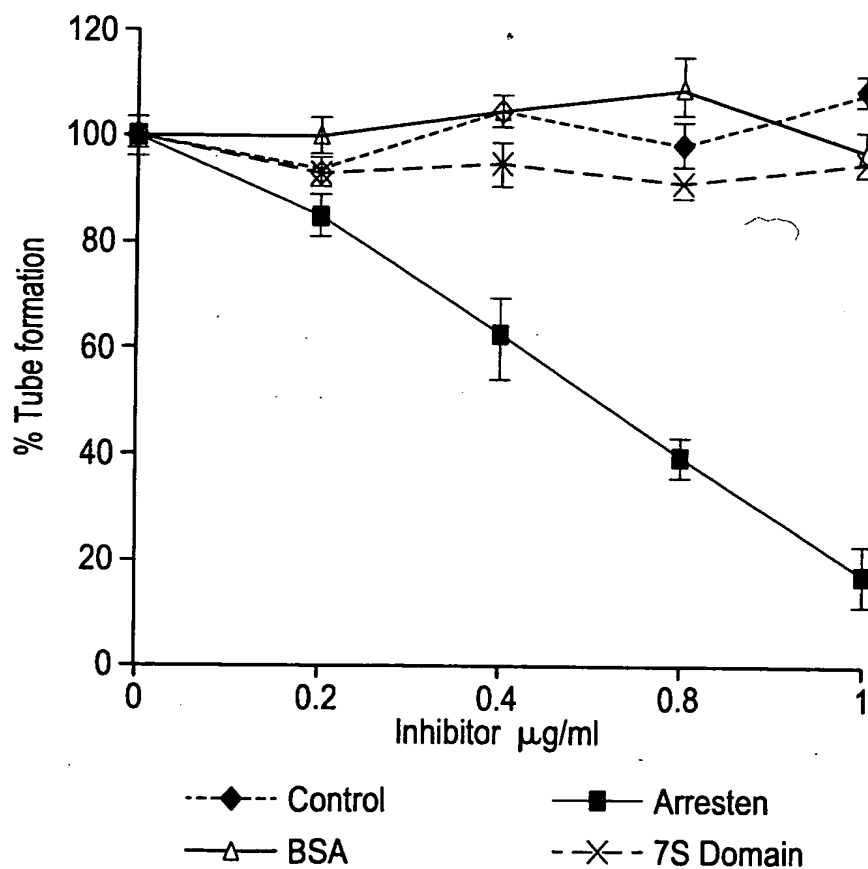


FIG. 7



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Inventors: Raghuram Kalluri

FIG. 8A

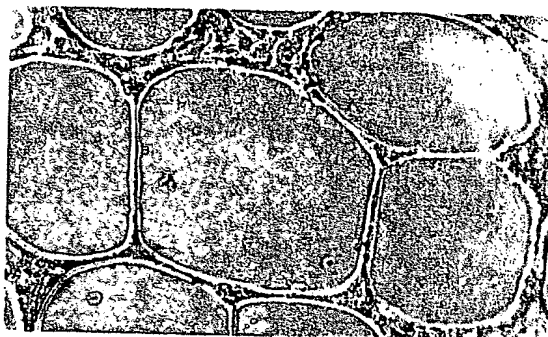


FIG. 8B



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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

FIG. 9A

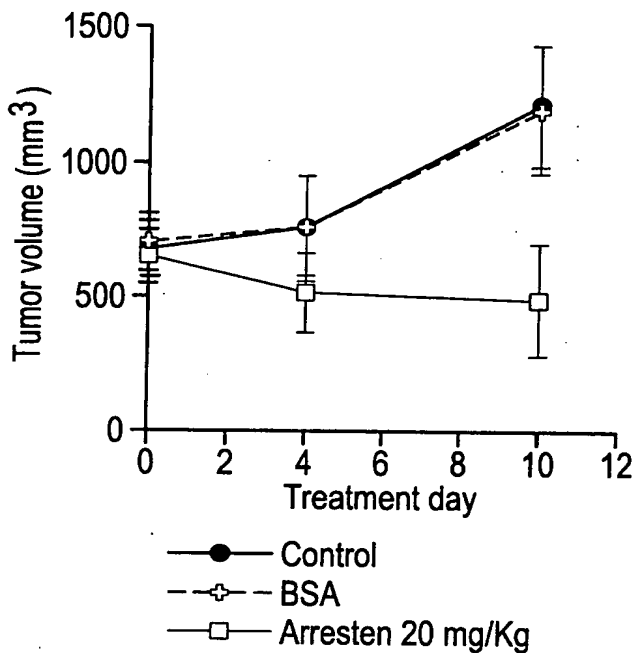


FIG. 9B

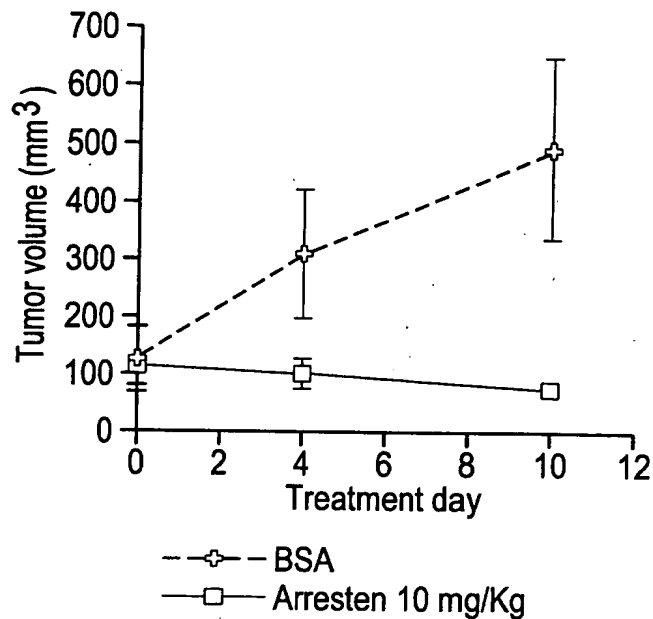


FIG. 9C

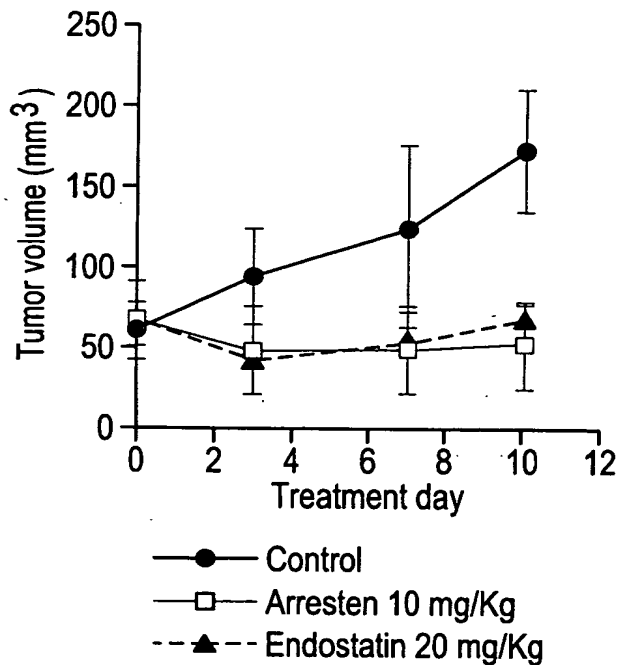
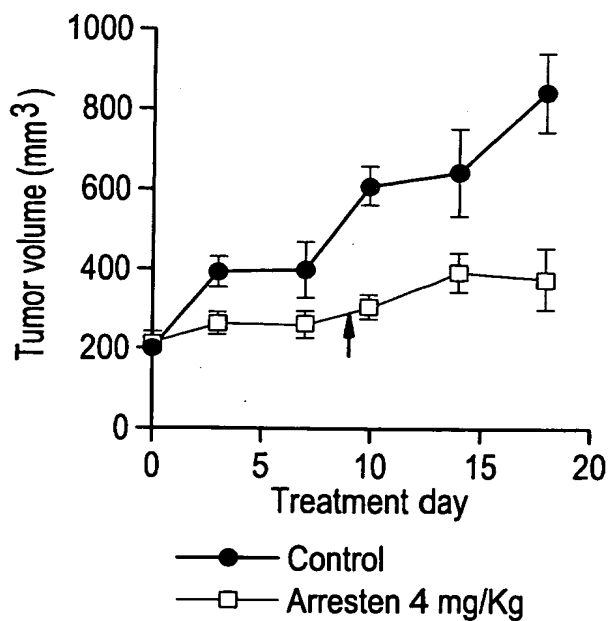


FIG. 9D



APPROVED	O.G. FIG.	
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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

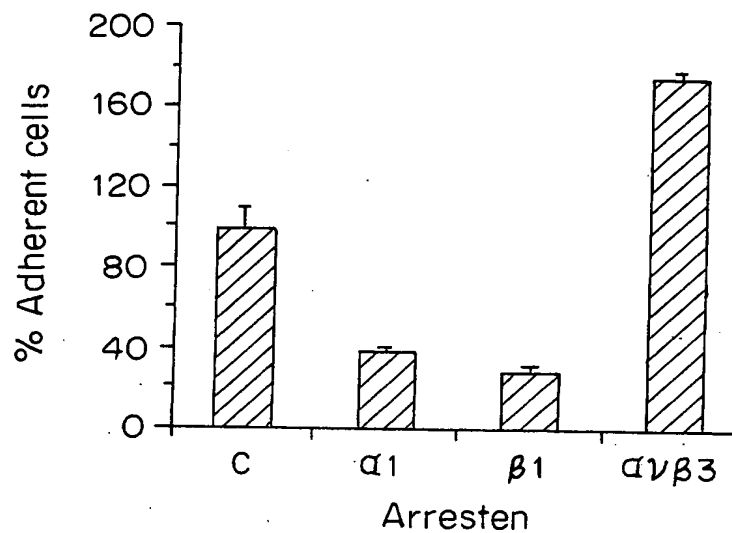


FIG. IOA

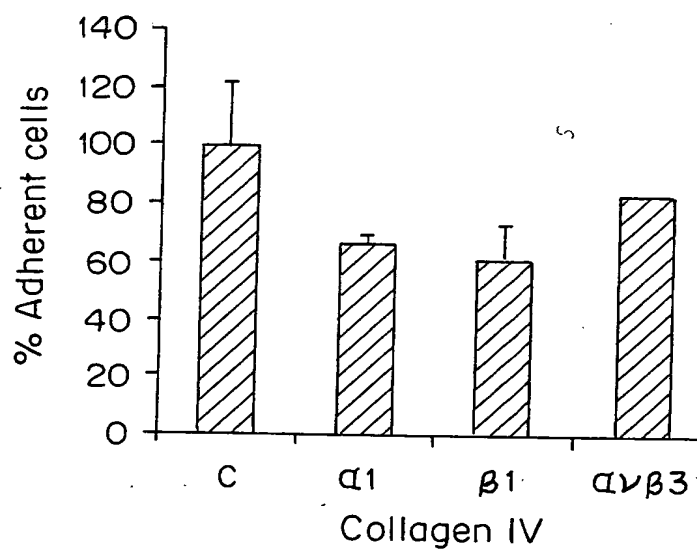


FIG. IOB

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Inventors: Raghuram Kalluri

FIG. 11A

pET22b(+) forward primer:

5'-CGGGATCCT GTC AGC ATC GGC TAC CTC-3' (SEQ ID NO:7)

pET22b(+) reverse primer:

5'-CCCAAGCTT CAG GTT CTT CAT GCA CAC-3' (SEQ ID NO:8)

pPICZαA forward primer:

5'-TTCGGAATTC GTC AGC ATC GGC TAC CTC CTG-3' (SEQ ID NO:17)

pPICZαA reverse primer:

5'-GGGGTACCCC CAG GTT CTT CAT GCA CAC CTG G-3' (SEQ ID NO:18)

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      5      10      15      20      25      30      35      40      45
gtc agc atc ggc tac ctg gtg aag cac agc cag acg gac cag
      50      55      60      65      70      75      80      85      90
gag ccc atg tgc ccg gtg ggc atg aac aaa ctg tgg agt gga tac
      95     100     105     110     115     120     125     130     135
agc ctg ctg tac ttc gag ggc cag gag aag gcg cac aac cag gac
     140     145     150     155     160     165     170     175     180
ctg ggg ctg gcg ggc tcc tgc ctg gcg cgg ttc agc acc atg ccc
     185     190     195     200     205     210     215     220     225
ttc ctg tac tgc aac cct ggt gat gtc tgc tac tat gcc agc cgg
     230     235     240     245     250     255     260     265     270
aac gac aag tcc tac tgg ctg tct acc act gcg ccg ctg ccc atg
     275     280     285     290     295     300     305     310     315
atg ccc gtg gcc gag gac gag atc aag ccc tac atc agc cgc tgt
     320     325     330     335     340     345     350     355     360
tct gtg tgt gag gcc ccg gcc atc gcc atc gcg gtc cac agt cag
     365     370     375     380     385     390     395     400     405
gat gtc tcc atc cca cac tgc cca gct ggg tgg cgg agt ttg tgg
     410     415     420     425     430     435     440     445     450
atc gga tat tcc ttc ctg atg cac acg gcg gcg gga gac gaa ggc
     455     460     465     470     475     480     485     490     495
ggt ggc caa tca ctg gtg tca ccg ggc agc tgt cta gag gac ttc
     500     505     510     515     520     525     530     535     540
cgc gcc aca cca ttc atc gaa tgc aat gga ggc cgc ggc acc tgc
     545     550     555     560     565     570     575     580     585
cac tac tac gcc aac aag tac agc ttc tgg ctg acc acc att ccc
     590     595     600     605     610     615     620     625     630
gag cag agc ttc cag ggc tcg ccc tcc gcc gac acg ctg aag gcc
     635     640     645     650     655     660     665     670     675
ggc ctg atc cgc aca cac atc agc cgc tgc cag gtg tgc atg aag
     680
aac ctg tga      (SEQ ID NO:5)

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Inventors: Raghuram Kalluri

FIG. 11B

	5	10	15	20	25	30	35	40	45
VSI	Gyl	LVK	HSQ	TDQ	EPM	CPV	GMN	KLW	SGY
							SLL	YFE	GQE
								KAH	NQD
	50	55	60	65	70	75	80	85	90
LGL	AGS	CLA	RFS	TMP	FLY	CNP	GDV	CYY	ASR
							NDK	SYW	LST
								TAP	LPM
	95	100	105	110	115	120	125	130	135
MPV	AED	EIK	PYI	SRC	SVC	EAP	AIA	IAV	HSQ
							DVS	IPH	CPA
								GWR	SLW
	140	145	150	155	160	165	170	175	180
IGY	SFL	MHT	AAG	DEG	GGQ	SLV	SPG	SCL	EDF
							RAT	PFI	ECN
								GGR	GTC
	185	190	195	200	205	210	215	220	225
HYY	ANK	YSF	WLT	TIP	EQS	FQG	SPS	ADT	LKA
							GLI	RTH	ISR
								CQV	CMK

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NL (SEQ ID NO:6)

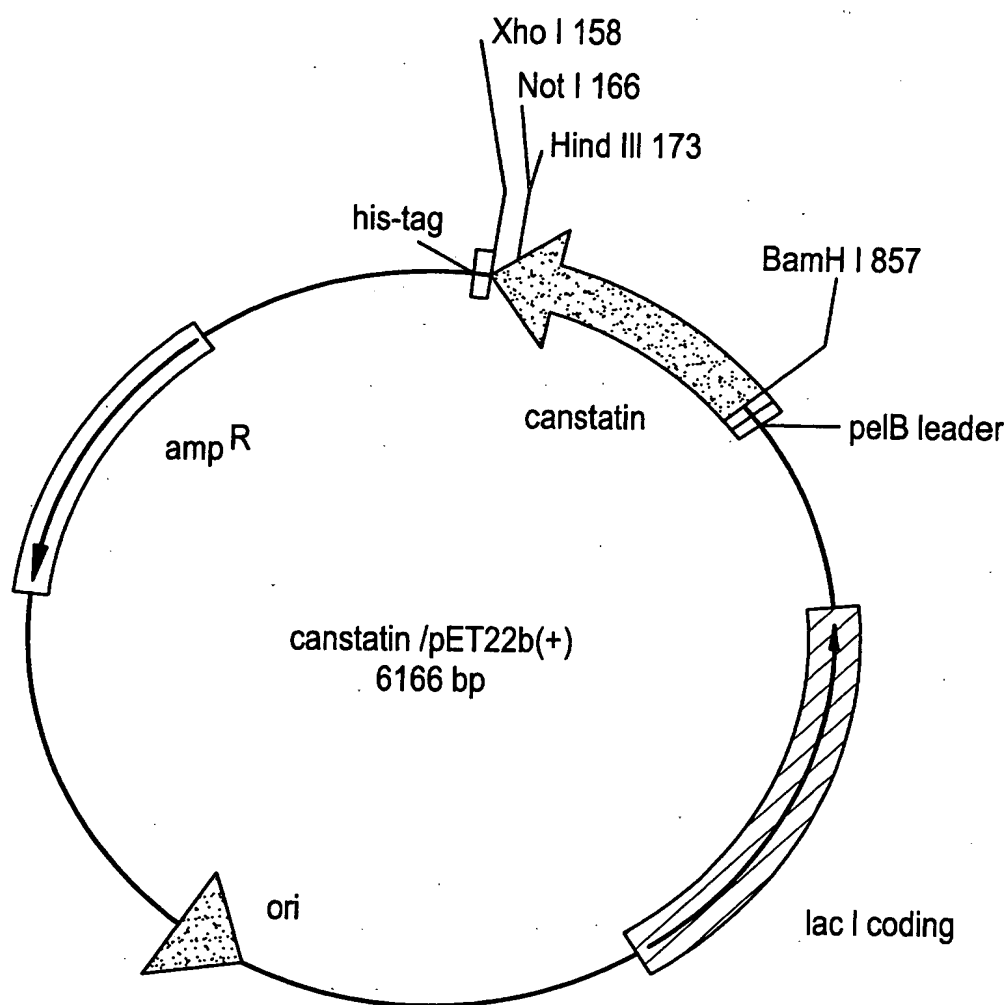
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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

FIG. 12



Forward primer: 5'-cgggatcctgtcagcatcggctacctc-3'

Reverse primer: 5'-cccaagcttcaggttcttcatgcacac-3'

FIG. 13A

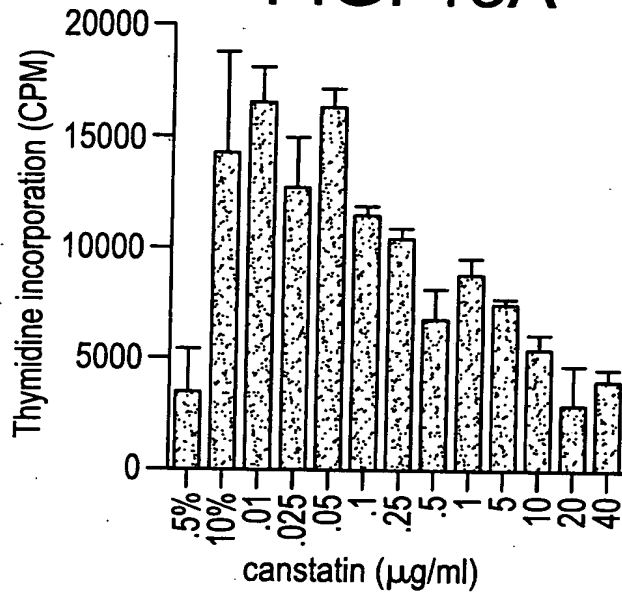
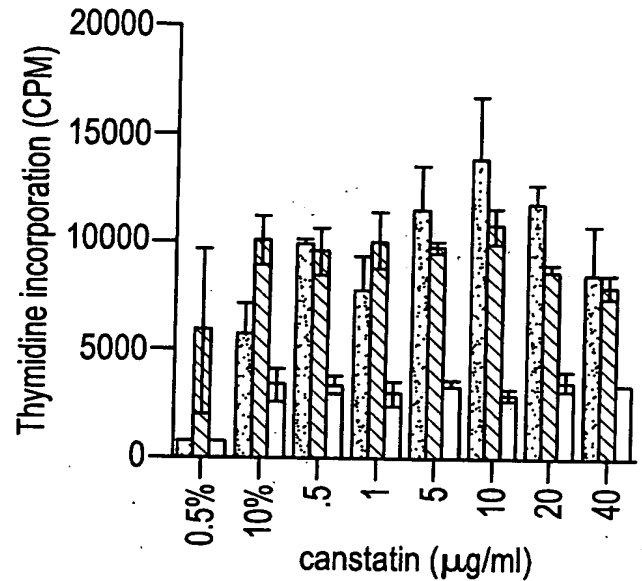


FIG. 13B



■ 786-0
 ▨ PC-3
 □ HEK 293

FIG. 13C

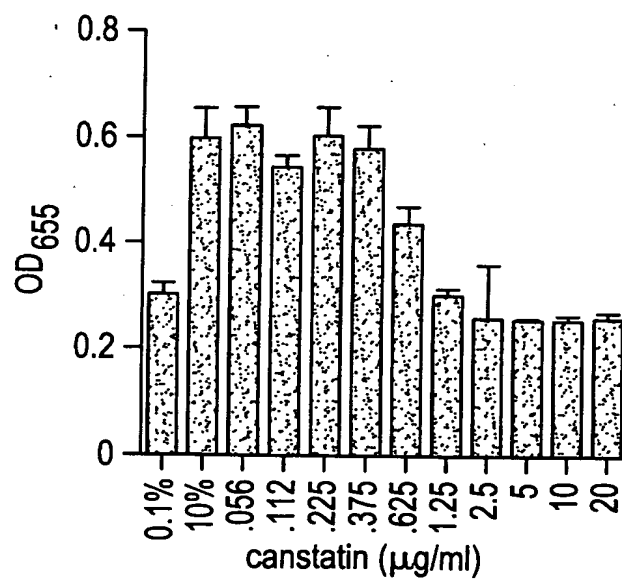
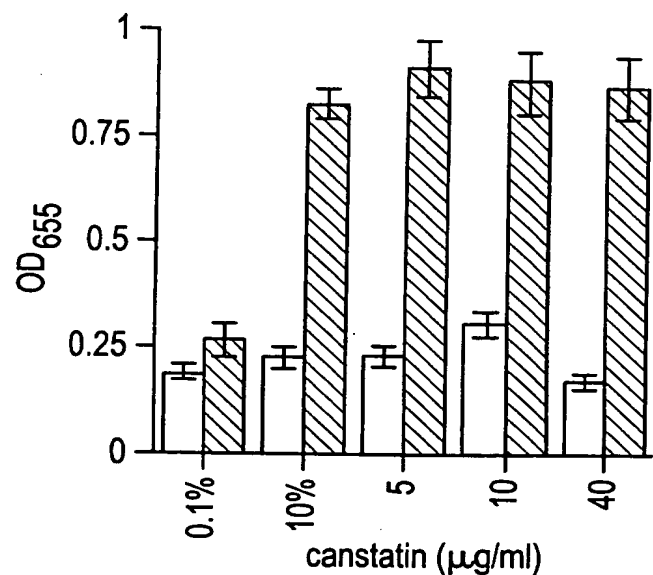


FIG. 13D



□ HEK 293
 ▨ PC-3

FIG. 14

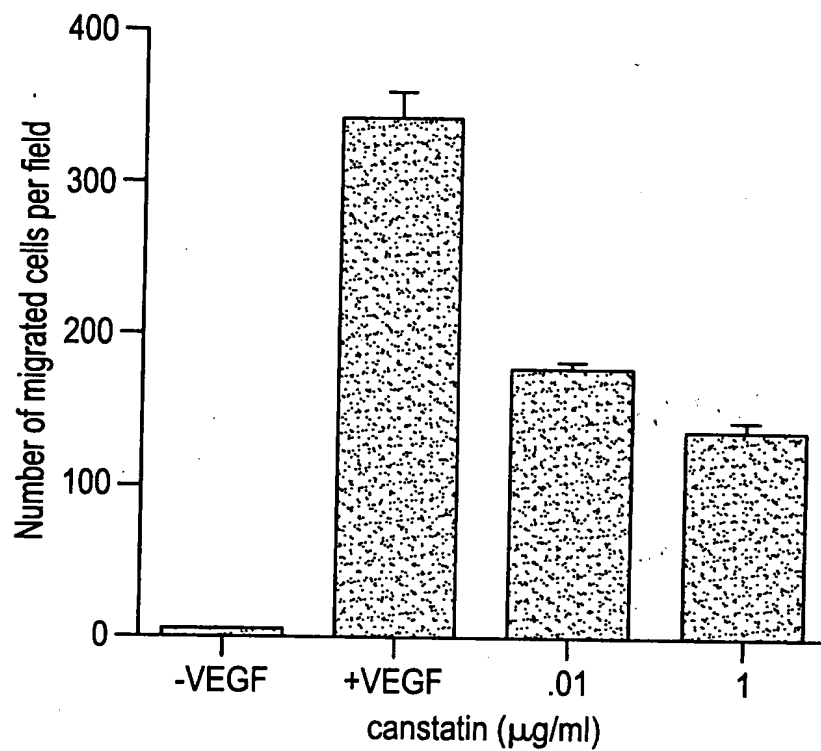
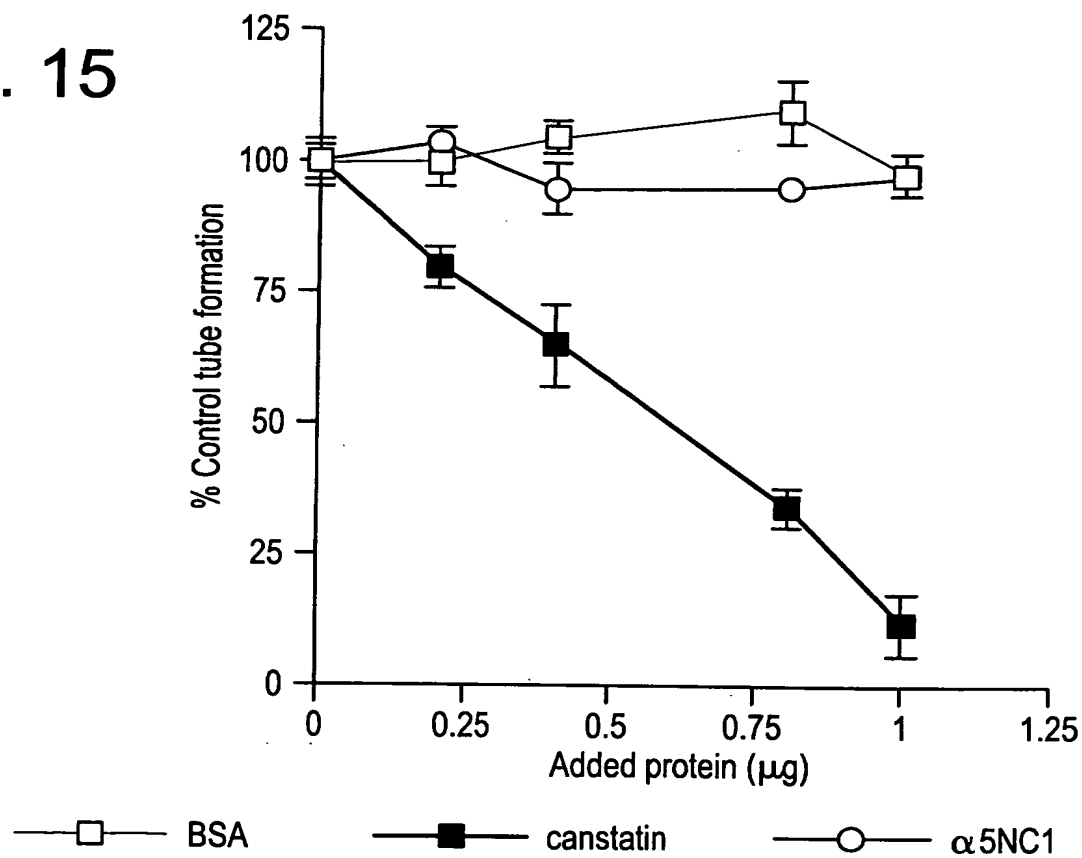


FIG. 15



APPROVED	O.G. FIG.	
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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

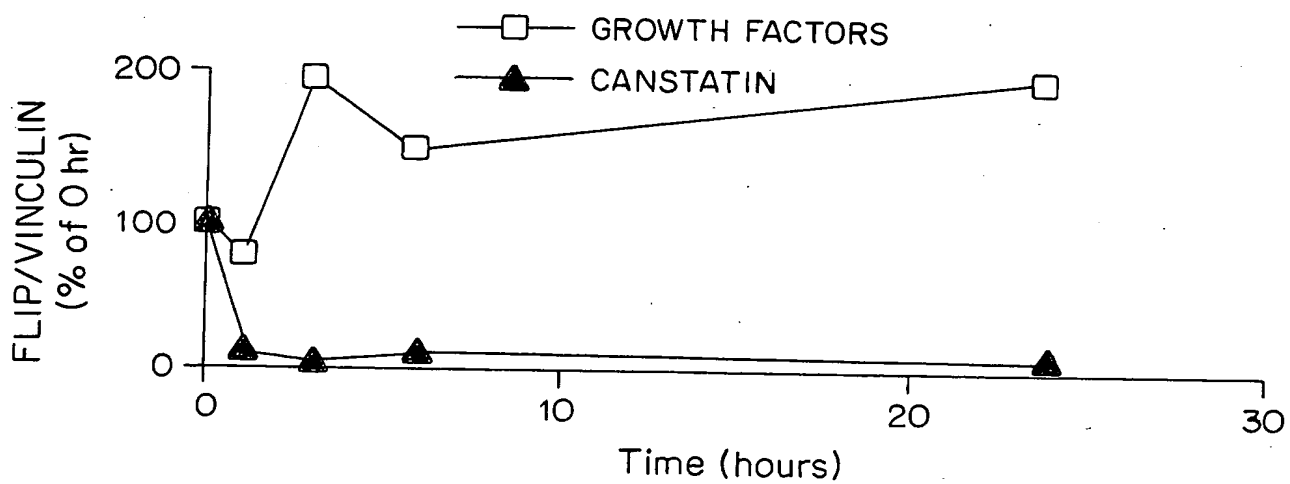


FIG. 16

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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

FIG. 17A

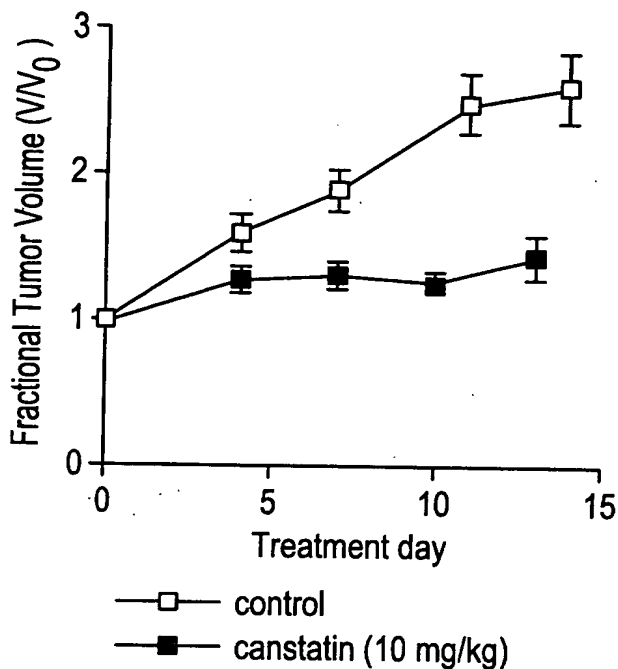


FIG. 17B

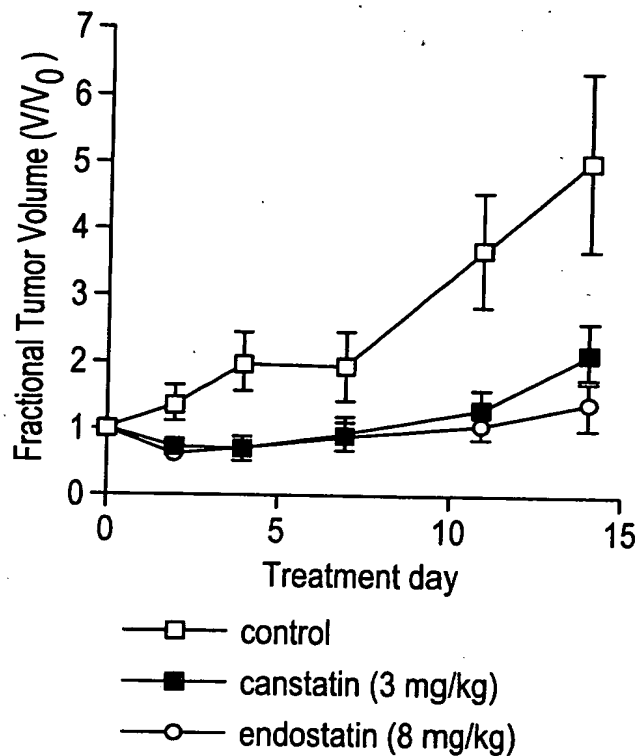


FIG. 17C

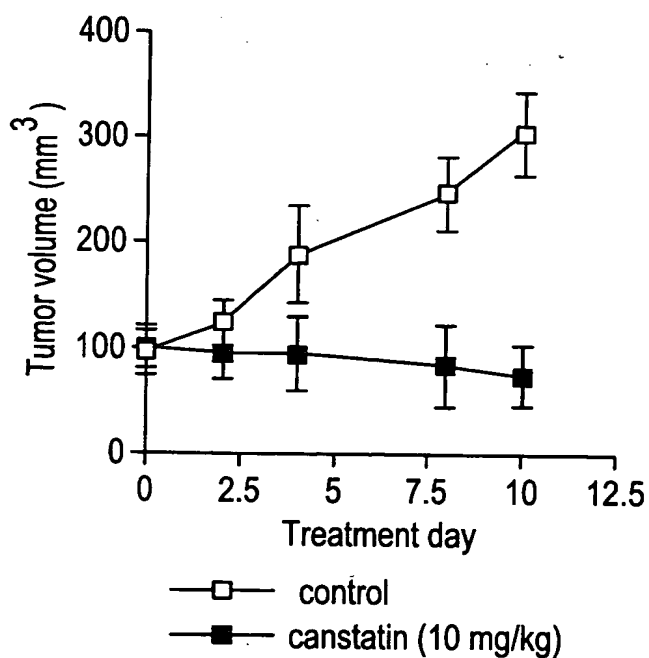
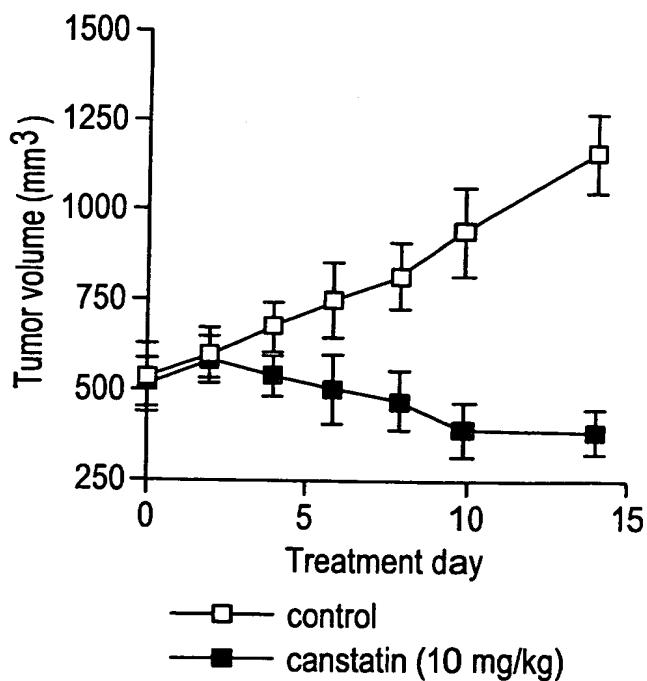


FIG. 17D



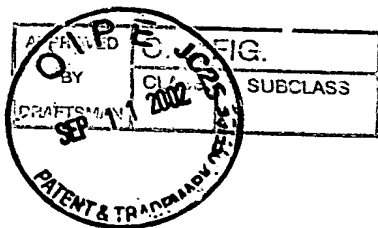


FIG. 18A

pET22b(+) forward primer:

5'-CGG GAT CCG GGT TTG AAA GGA AAA CGT-3' (SEQ ID NO:11)

pET22b(+) reverse primer:

5'-CCC AAG CTT TCA GTG TCT TTT CTT CAT-3' (SEQ ID NO:12)

5	10	15	20	25	30	35	40	45						
<u>ggt ttg</u>	<u>aaa gga</u>	<u>aaa cgt</u>	gga	gac	agt	gga	tca	cct	gca	acc	tgg			
50	55	60	65	70	75	80	85	90						
aca	acg	aga	ggc	ttt	gtc	ttc	acc	cga	cac	agt	caa	acc	aca	gca
95	100	105	110	115	120	125	130	135						
att	cct	tca	tgt	cca	gag	ggg	aca	gtg	cca	ctc	tac	agt	ggg	ttt
140	145	150	155	160	165	170	175	180						
tct	ttt	ctt	ttt	gta	caa	gga	aat	caa	cga	gcc	cac	gga	caa	gac
185	190	195	200	205	210	215	220	225						
ctt	gga	act	ctt	ggc	agc	tgc	ctg	cag	cga	ttt	acc	aca	atg	cca
230	235	240	245	250	255	260	265	270						
ttc	tta	ttc	tgc	aat	gtc	aat	gat	gta	tgt	aat	ttt	gca	tct	cga
275	280	285	290	295	300	305	310	315						
aat	gat	tat	tca	tac	tgg	ctg	tca	aca	cca	gct	ctg	atg	cca	atg
320	325	330	335	340	345	350	355	360						
aac	atg	gct	ccc	att	act	ggc	aga	gcc	ctt	gag	cct	tat	ata	agc
365	370	375	380	385	390	395	400	405						
aga	tgc	act	gtt	tgt	gaa	ggc	cct	gag	atc	gcc	ata	gcc	gtt	cac
410	415	420	425	430	435	440	445	450						
agc	caa	acc	act	gac	att	cct	cca	tgt	cct	cac	ggc	tgg	att	tct
455	460	465	470	475	480	485	490	495						
ctc	tgg	aaa	gga	ttt	tca	ttc	atc	atg	ttc	aca	agt	gca	ggc	tct
500	505	510	515	520	525	530	535	540						
gag	ggc	acc	ggg	caa	gca	ctg	gcc	tcc	cct	ggc	tcc	tgc	ctg	gaa
545	550	555	560	565	570	575	580	585						
gaa	ttc	cga	gcc	agc	cca	ttt	cta	gaa	tgt	cat	gga	aga	gga	acg
590	595	600	605	610	615	620	625	630						
tgc	aac	tac	tat	tca	aat	tcc	tac	agt	ttc	tgg	ctg	gct	tca	tta
635	640	645	650	655	660	665	670	675						
aac	cca	gaa	aga	atg	ttc	aga	aag	cct	att	cca	tca	act	gtg	aaa
680	685	690	695	700	705	710	715	720						
gct	ggg	gaa	tta	gaa	aaa	ata	ata	agt	cgc	tgt	cag	gtg	tgc	<u>atg</u>
725	730	735												
<u>aaq</u>	<u>aaa</u>	<u>aga</u>	<u>cac</u>	<u>tga</u>	(SEQ ID NO:9)									

pET22b- α 3(IV) NC1 = nucleotides 1 through 732

Tumstatin 333 = nucleotides 1 through 372

Tumstatin 334 - nucleotide 373 through 732

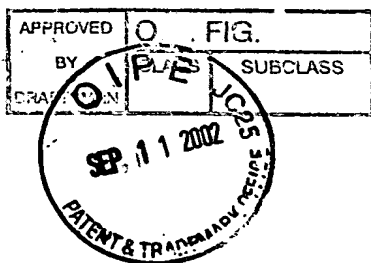


FIG. 18B

*
5 10 15 20 25 30 35 40 45
GLK GKR GDS GSP ATW TTR GFV FTR HSQ TTA IPS CPE GTV PLY SGF

50 55 60 65 70 75 80 85 90
SFL FVQ GNQ RAH GQD LGT LGS CLQ RFT TMP FLF CNV NDV CNF ASR

*+
95 100 105 110 115 120 125 130 135
NDY SYW LST PAL MPM NMA PIT GRA LEP YIS RCT VCE GPA IAI AVH

140 145 150 155 160 165 170 175 180
SQT TDI PPC PHG WIS LWK GFS FIM FTS AGS EGT GQA LAS PGS CLE

185 190 195 200 205 210 215 220 225
EFR ASP FLE CHG RGT CNY YSN SYS FWL ASL NPE RMF RKP IPS TVK

+
230 235 240 244
AGE LEK IIS RCQ VCM KKR H (SEQ ID NO:10)

pET22b α 3(IV) NC1 = residues 1 through 244

Tumstatin 333 = residues 1 through 124

Tumstatin 334 = residues 125 through 244

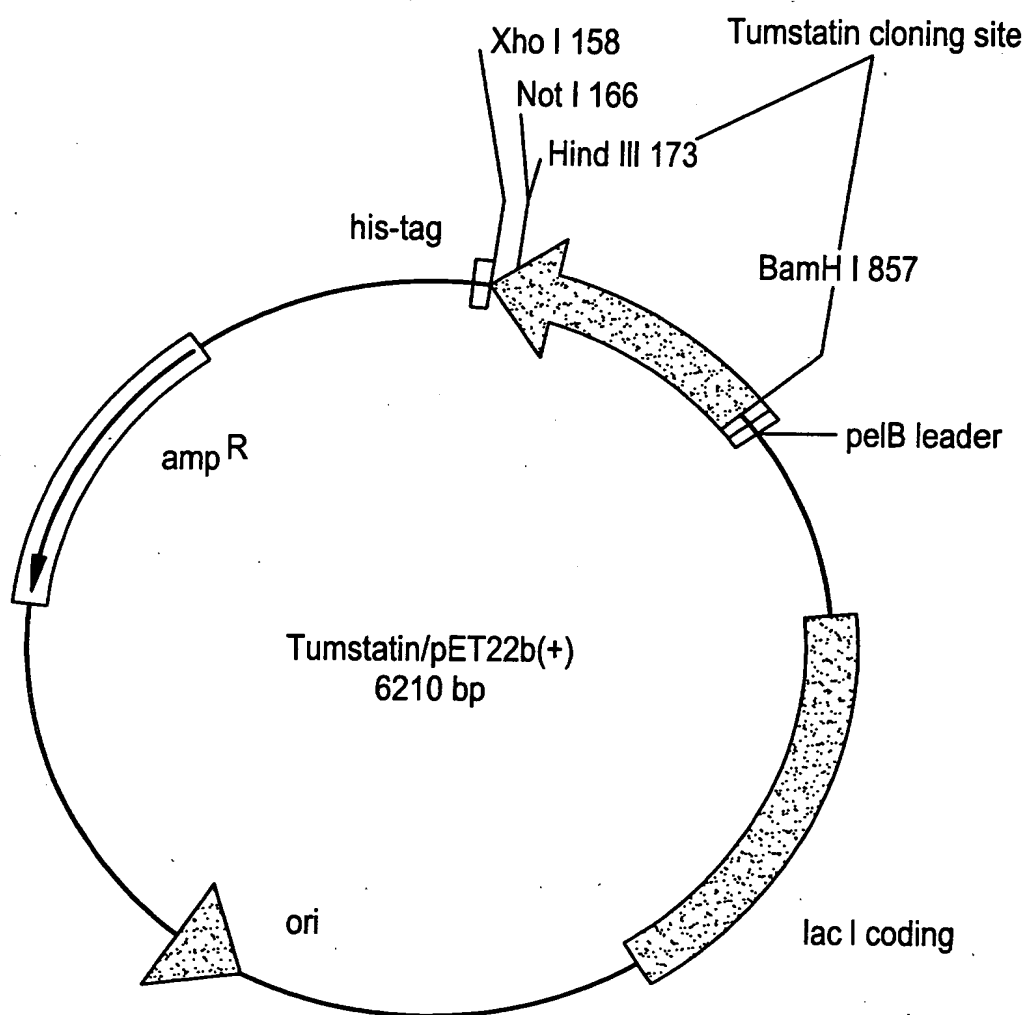
APPROVED	O.G. FIG.	
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Docket/App No.: 1440.1027-005

Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

FIG. 19



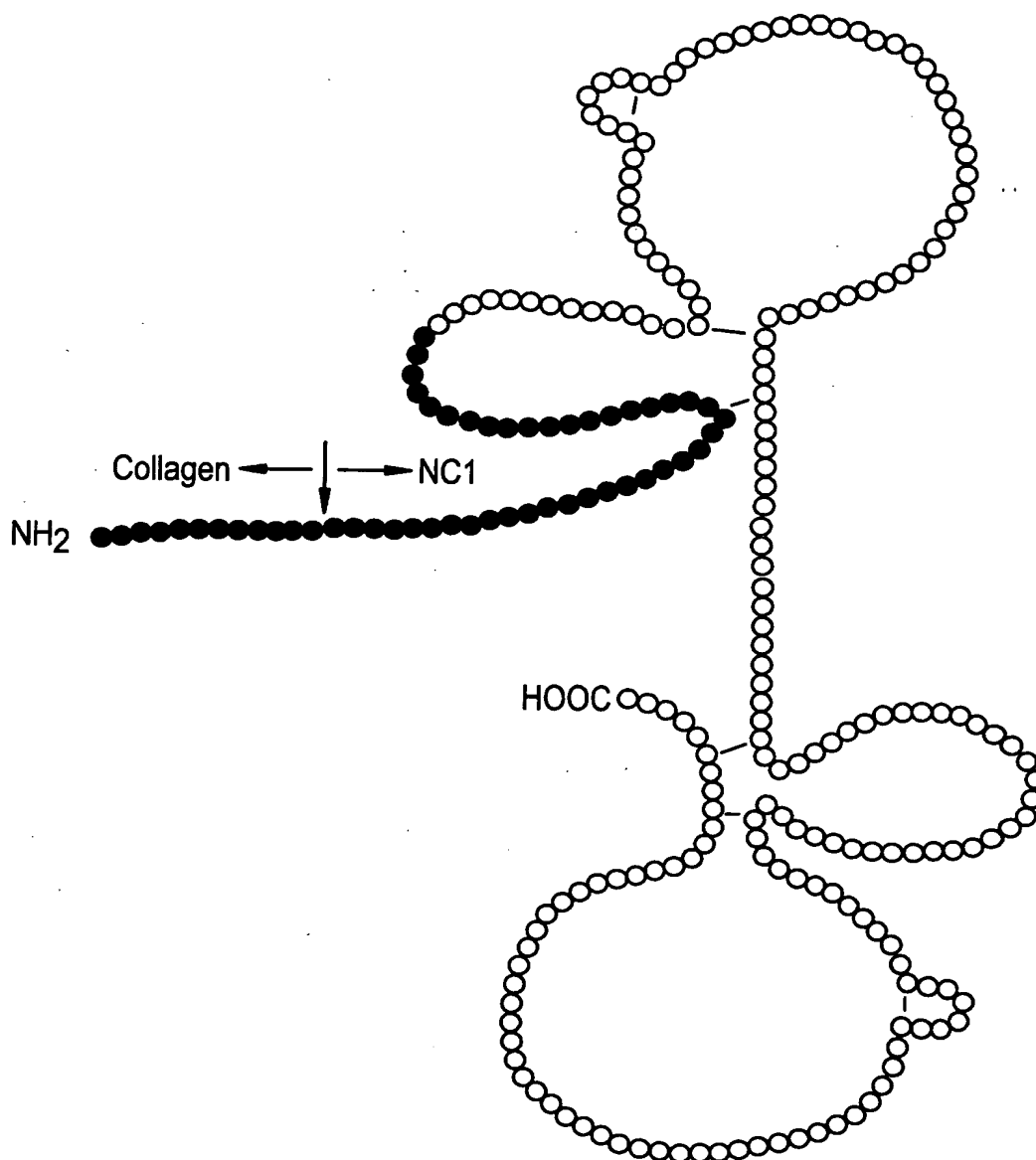
APPROVED	O.G. FIG.	
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Inventors: Raghuram Kalluri

FIG. 20



APPROVED	O.G. FIG.	
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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

FIG. 21A

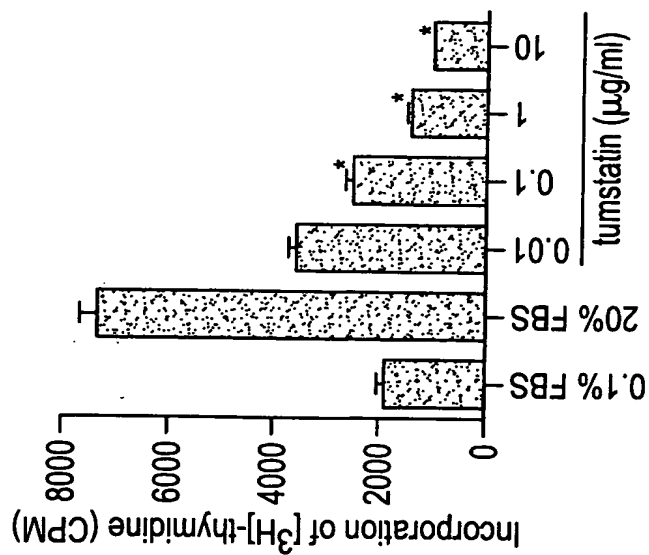


FIG. 21B

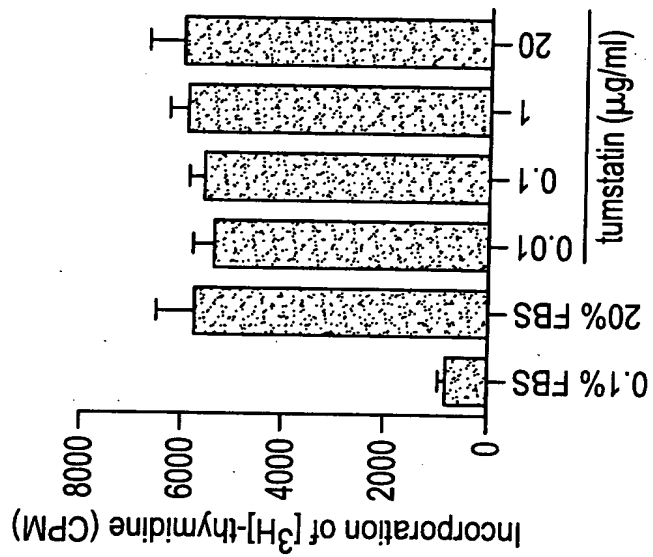
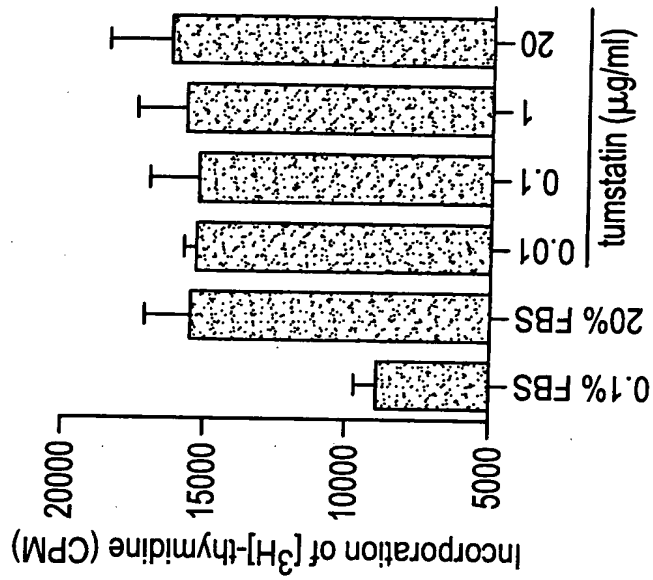


FIG. 21C



APPROVED	O.G. FIG.	
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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

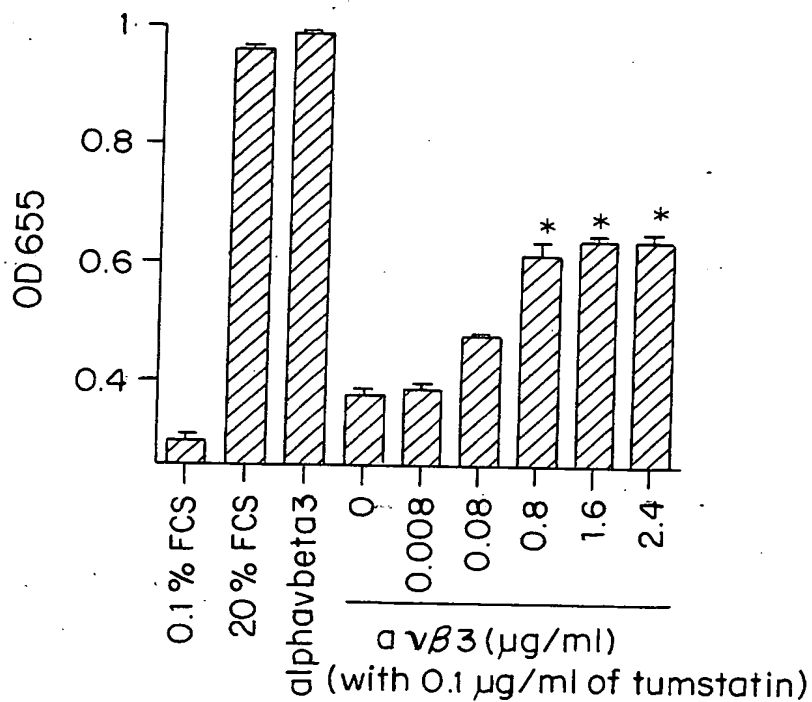


FIG. 22

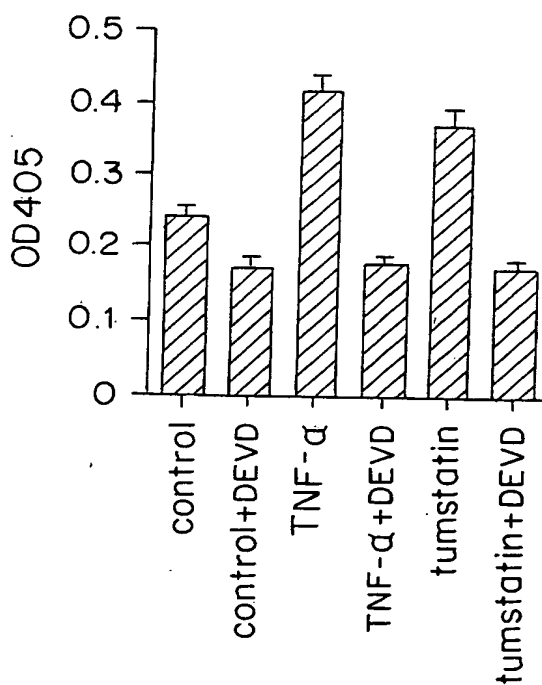


FIG. 23A

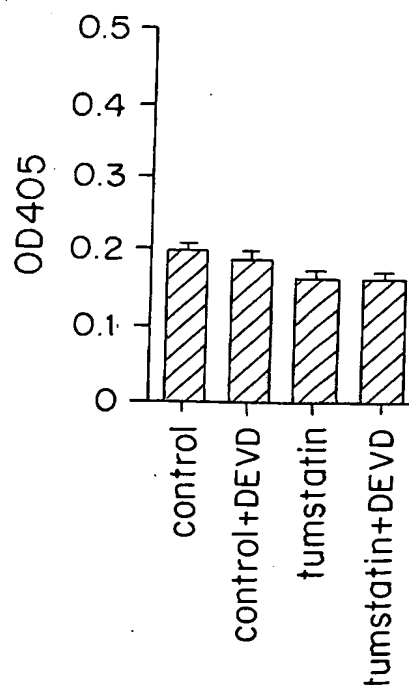


FIG. 23B

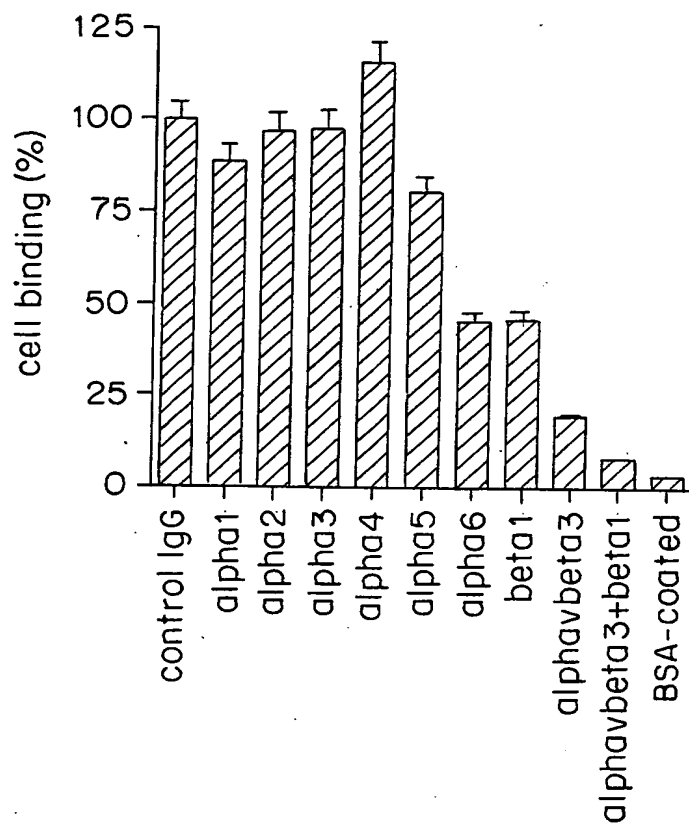


FIG. 24A

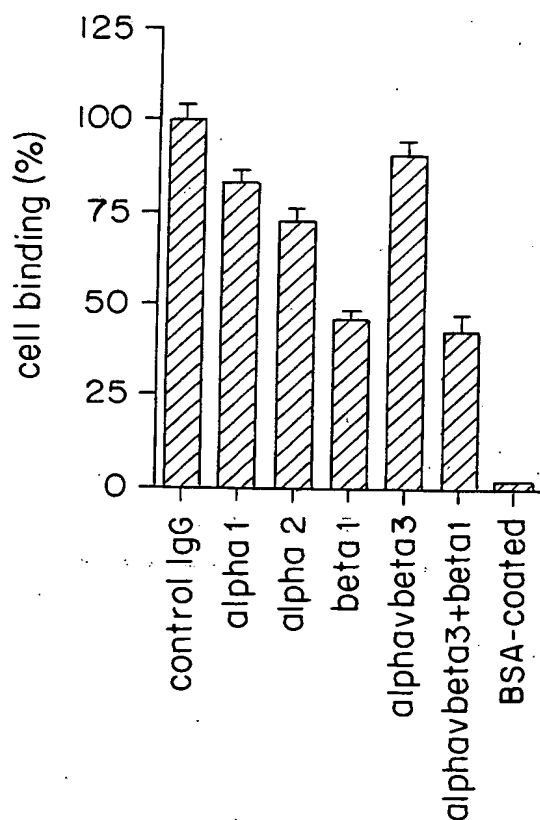


FIG. 24B

APPROVED	O.G. FIG.	
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Inventors: Raghuram Kalluri

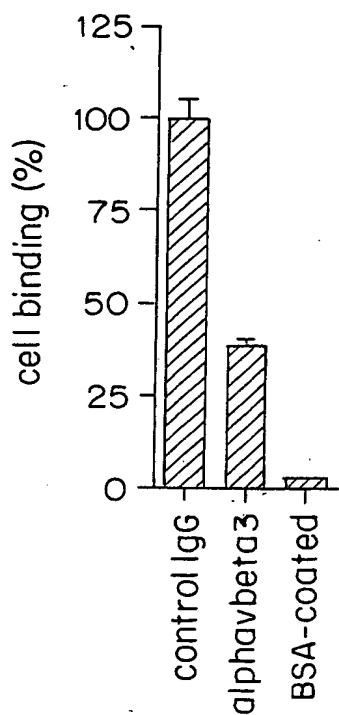


FIG. 24C

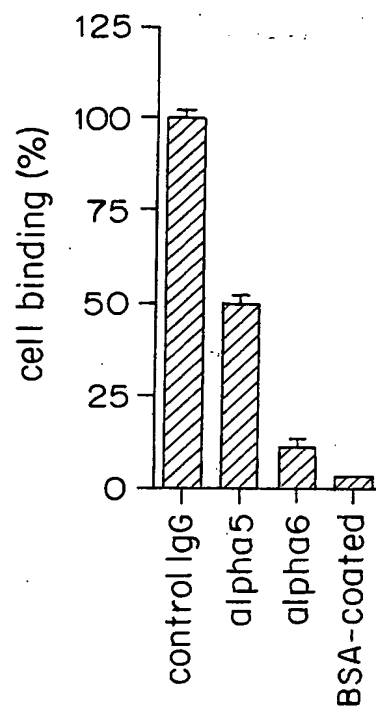


FIG. 24D

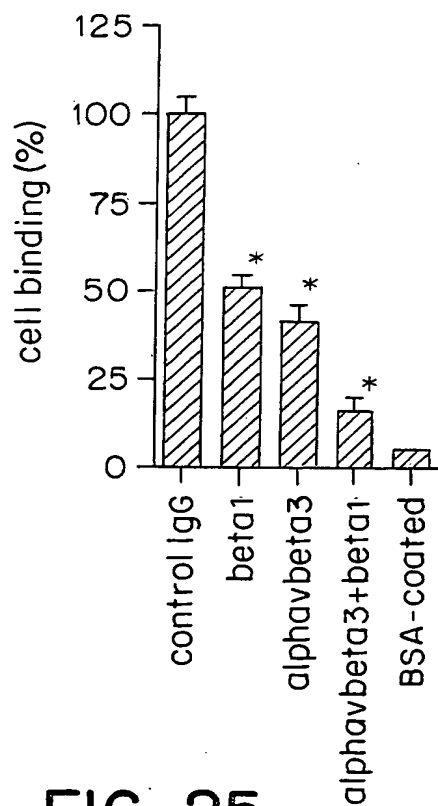


FIG. 25

APPROVED	O.G. FIG.	
BY	CLASS	SUBCLASS
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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

FIG. 26

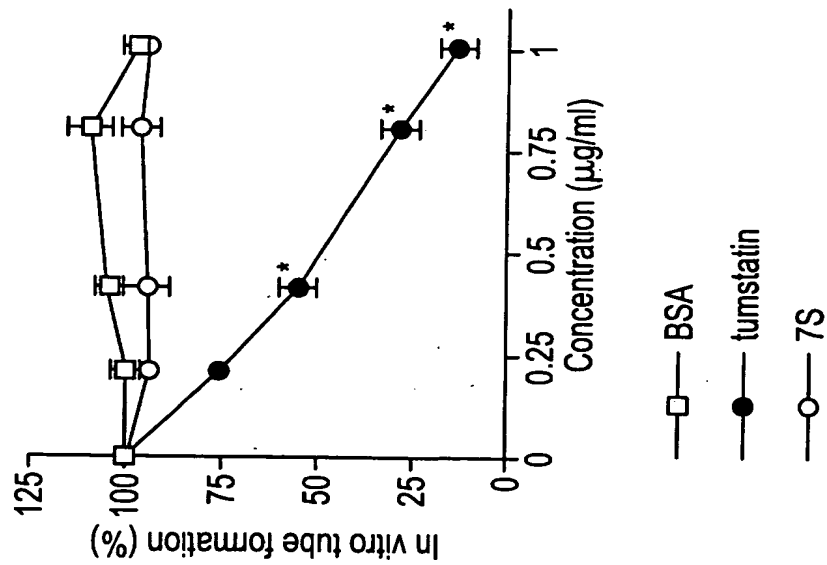


FIG. 27A

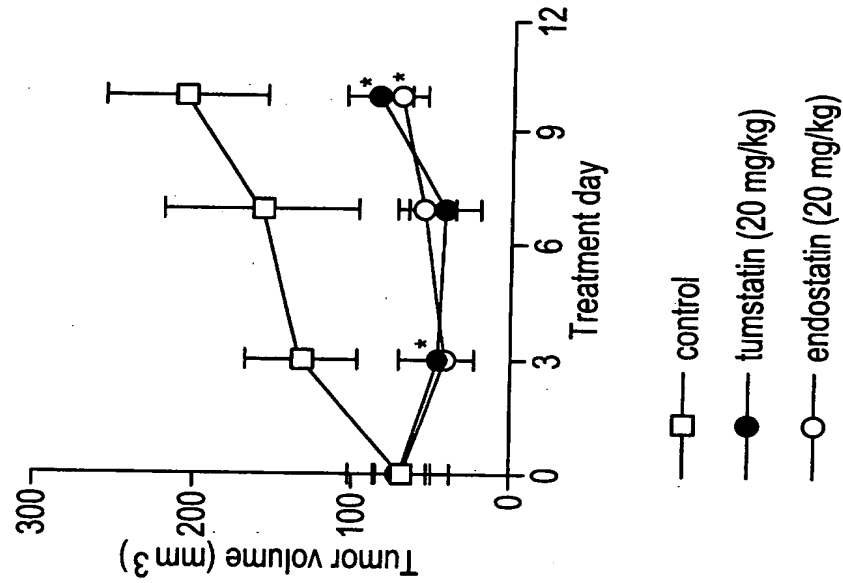
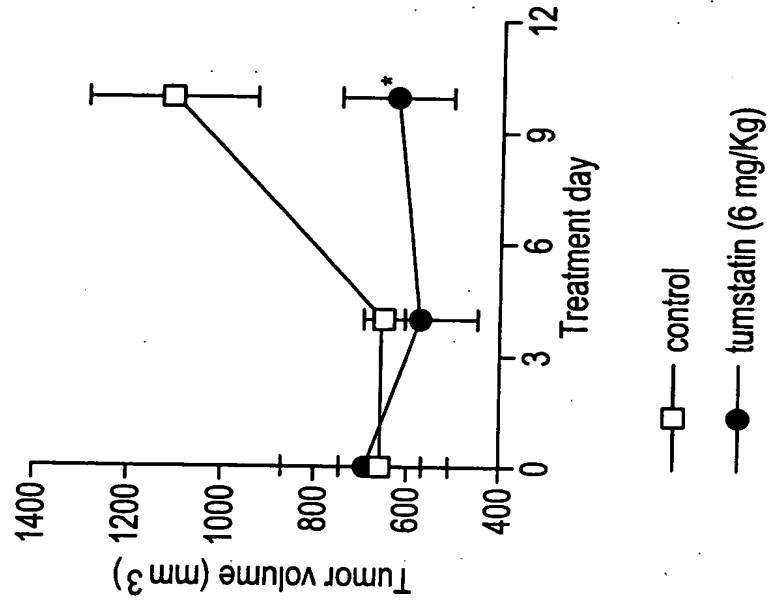


FIG. 27B



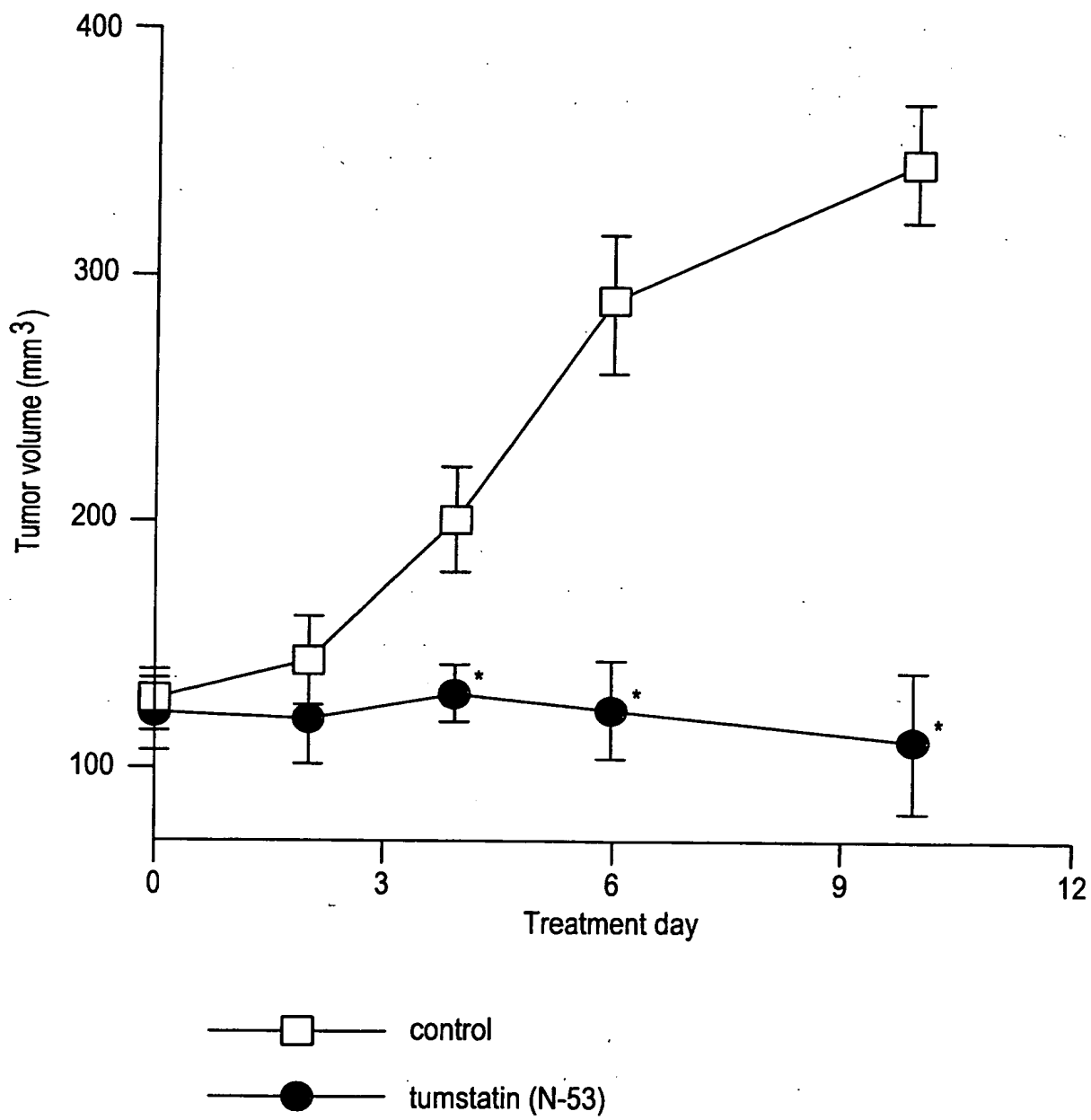
APPROVED	O.G. FIG.	
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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

FIG. 28



APPROVED	O.G. FIG.	
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Docket/App No.: 1440.1027-005

Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

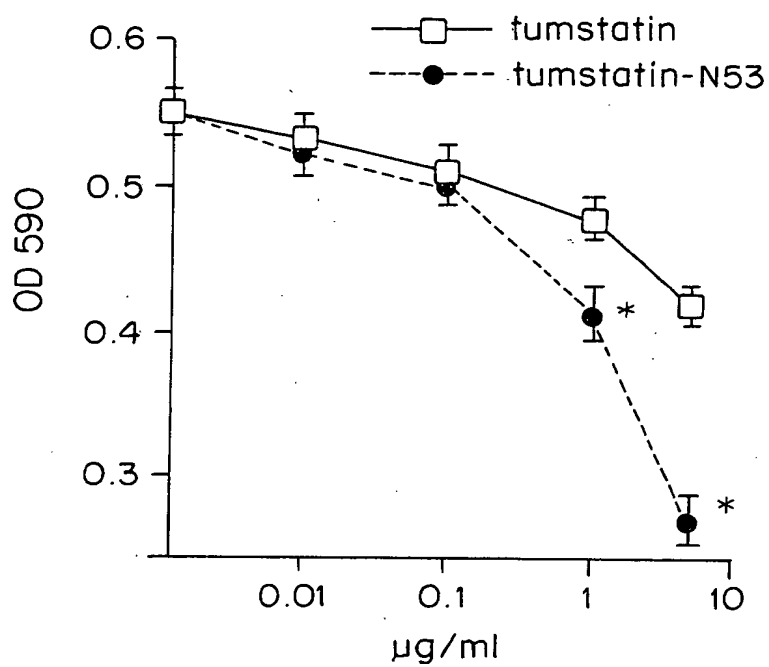


FIG. 29

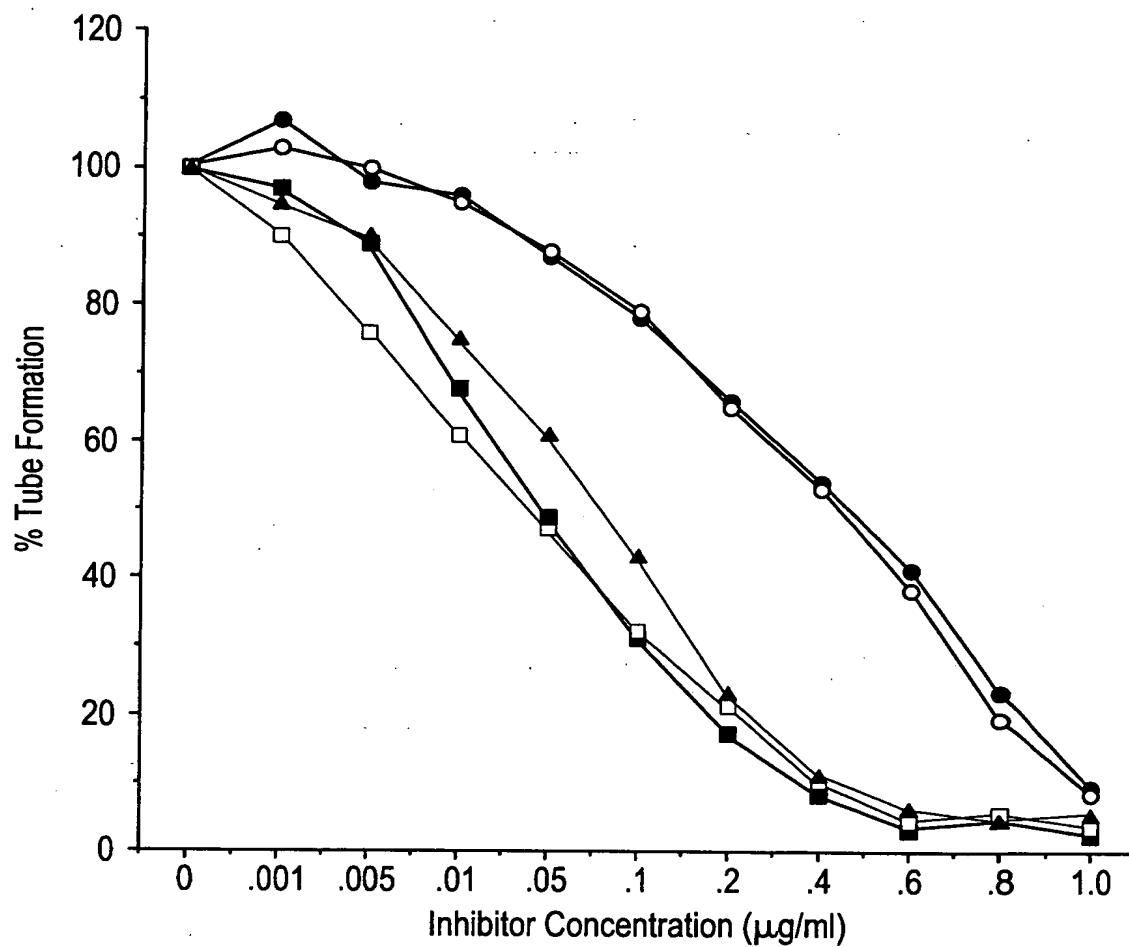
APPROVED	O.G. FIG.	
BY	CLASS	SUBCLASS
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Docket/App No.: 1440.1027-005

Title: Anti-Angiogenic Proteins and Fragments and Method of Use Thereof

Inventors: Raghuram Kalluri

FIG. 30



- Arresten
- Canstatin
- 12 kDa fragment of Arresten
- 8 kDa fragment of Arresten
- ▲— 10 kDa fragment of Canstatin

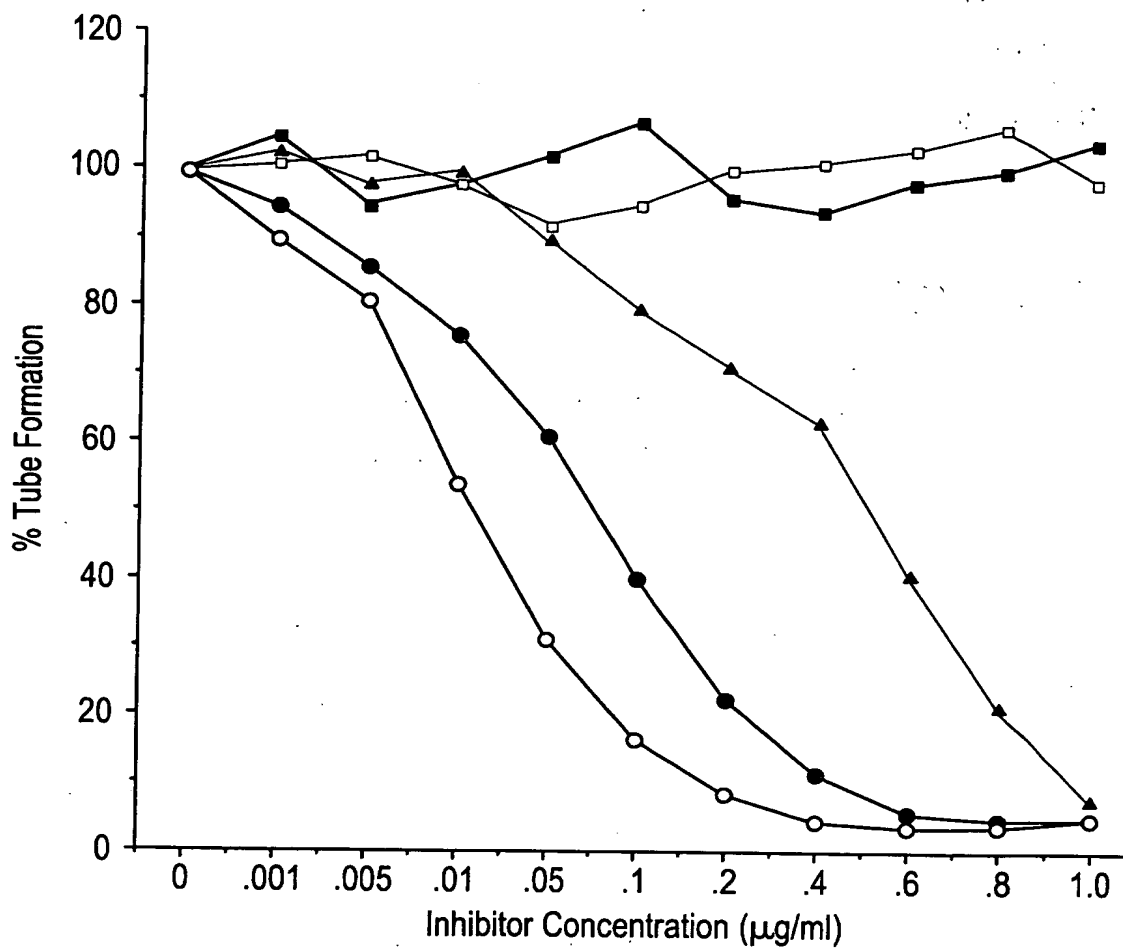
APPROVED	O.G. FIG.	
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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

FIG. 31



- Tumstatin Fragment 333
- Tumstatin Fragment 334
- BSA
- α6
- ▲— Tumstatin

APPROVED	O.G. FIG.	
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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

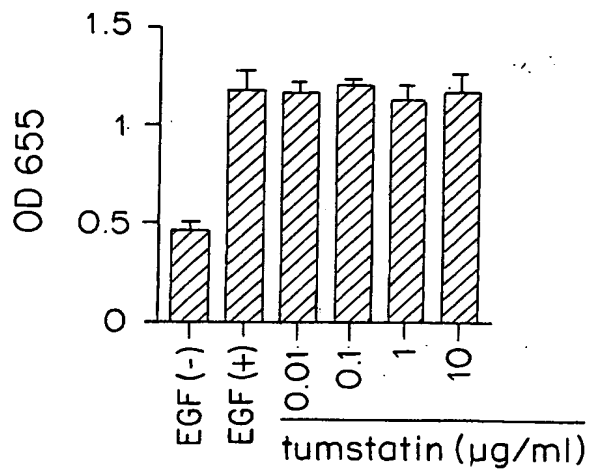


FIG. 32A

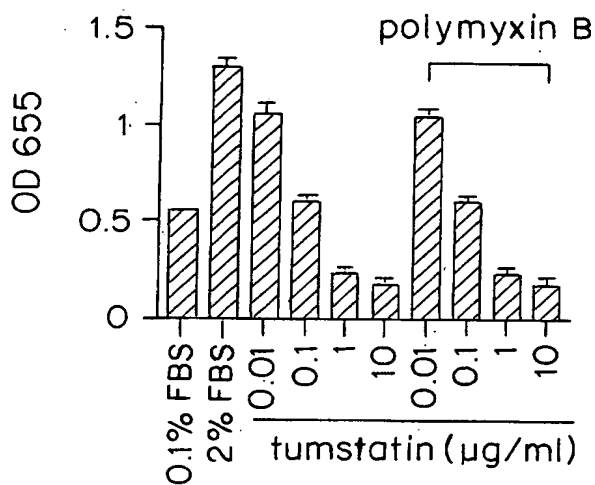


FIG. 32B

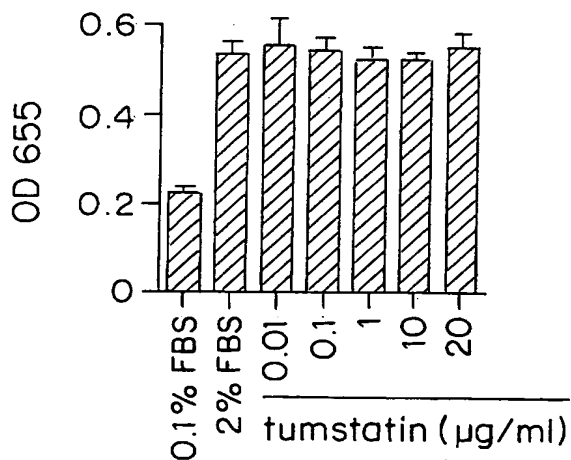


FIG. 32C

APPROVED	O.G. FIG.
BY	CLASS SUBCLASS
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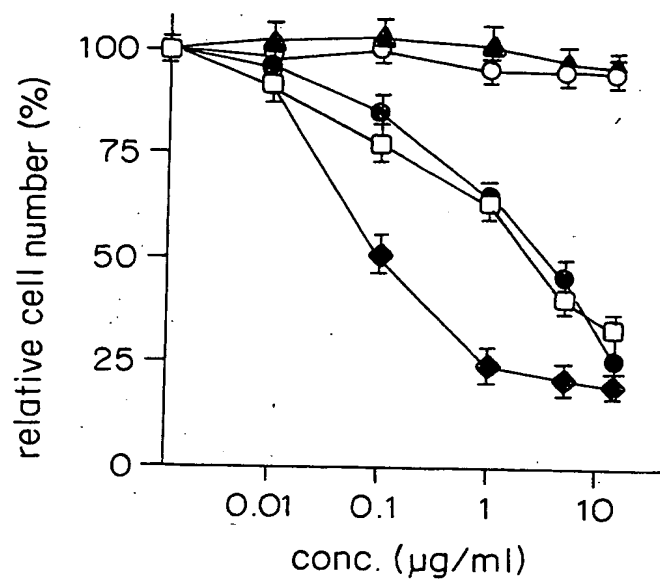


FIG. 33A

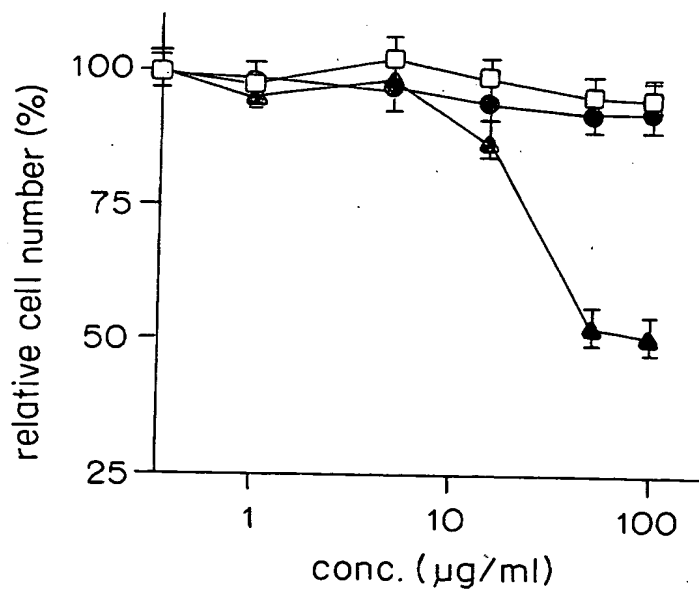


FIG. 33B

APPROVED	O.G. FIG.	
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Inventors: Raghuram Kalluri

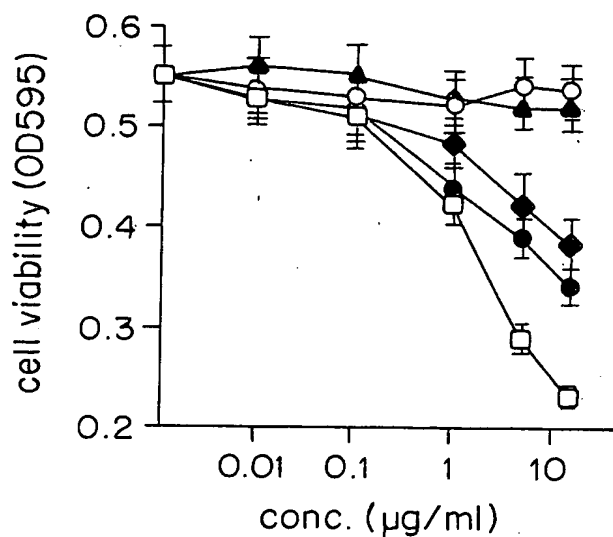


FIG. 34A

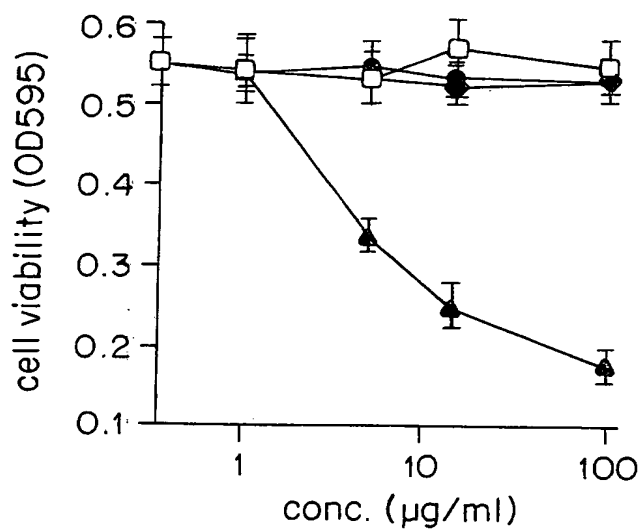


FIG. 34B

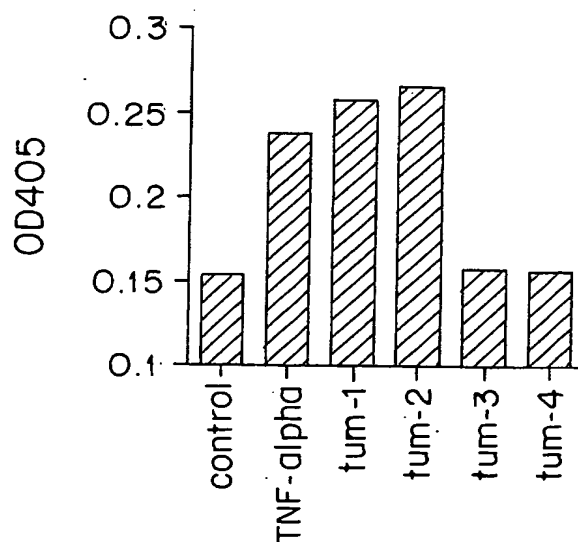


FIG. 35

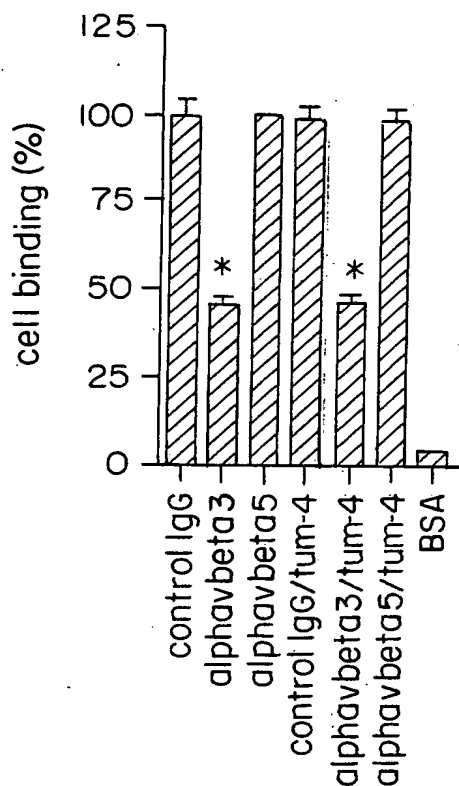


FIG. 36A

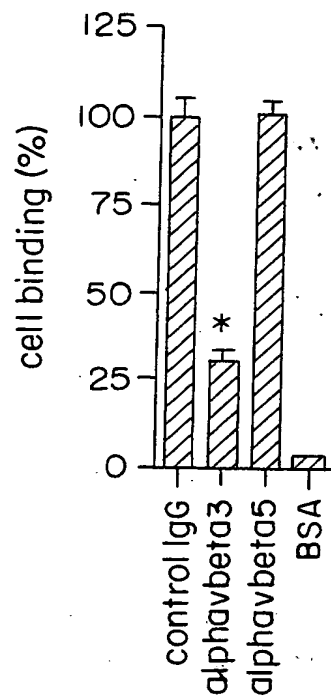


FIG. 36B

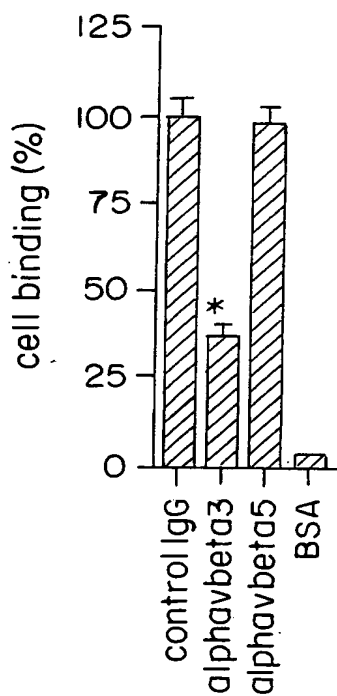


FIG. 36C

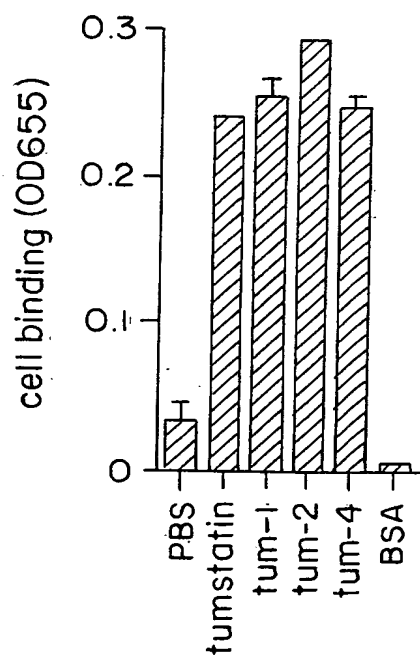


FIG. 37

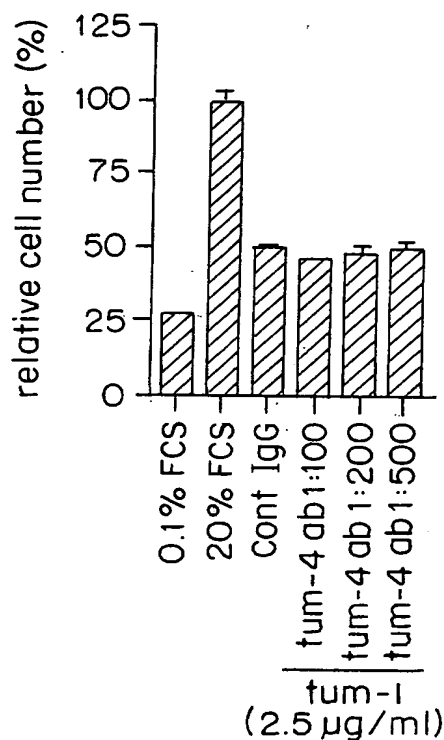


FIG. 38A

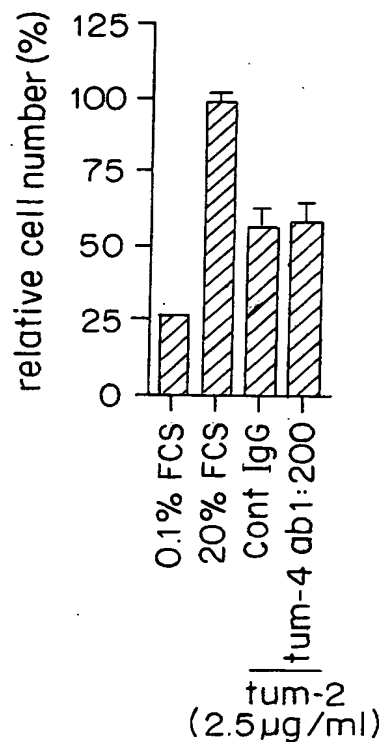


FIG. 38B

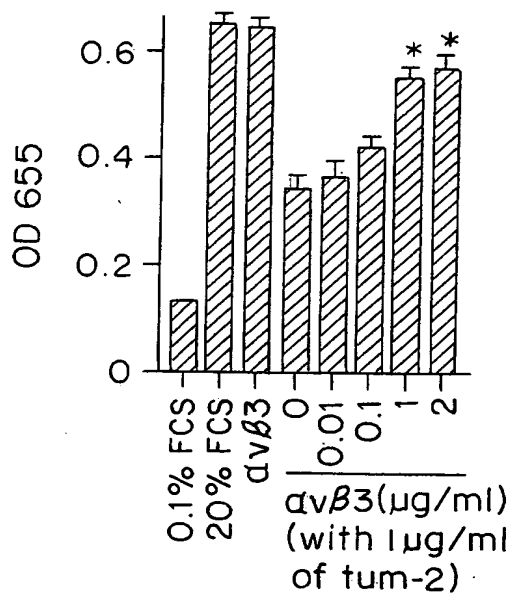


FIG. 38C

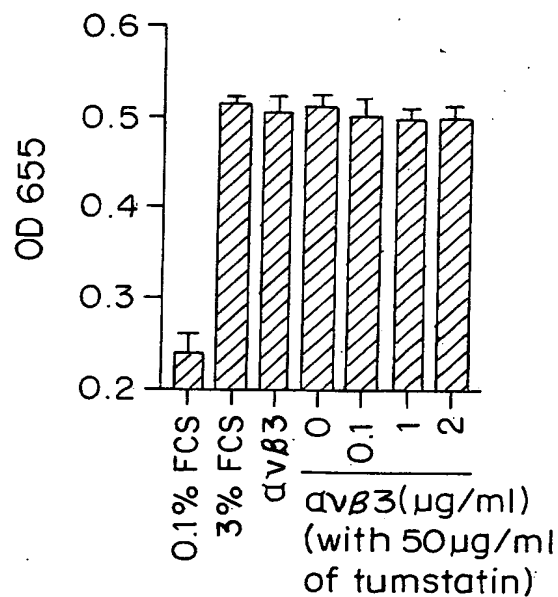


FIG. 38D

APPROVED	O.G. FIG.	
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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

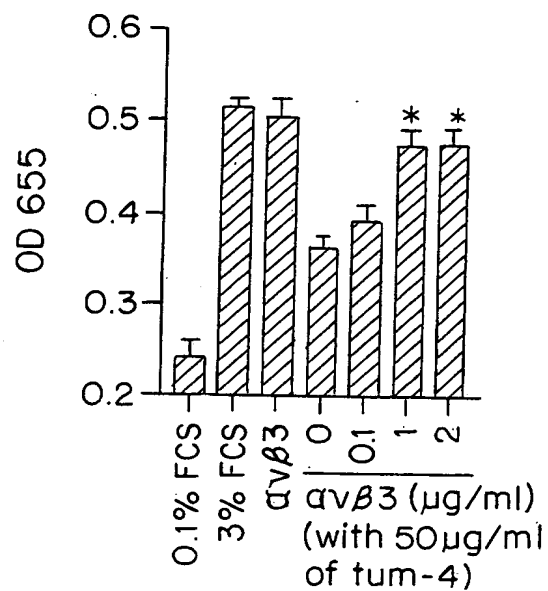


FIG. 38E

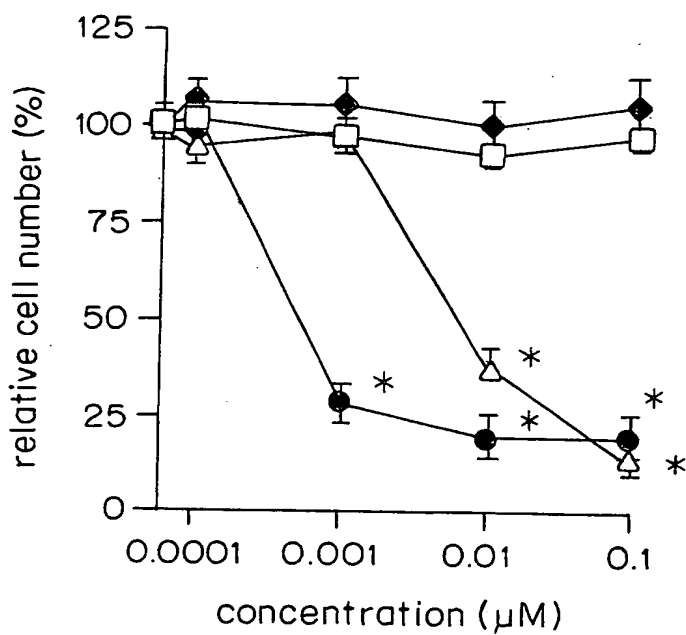


FIG. 39

APPROVED	O.G. FIG.	
BY	CLASS	SUBCLASS
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Title: Anti-Angiogenic Proteins and Fragments and Methods of Use Thereof

Inventors: Raghuram Kalluri

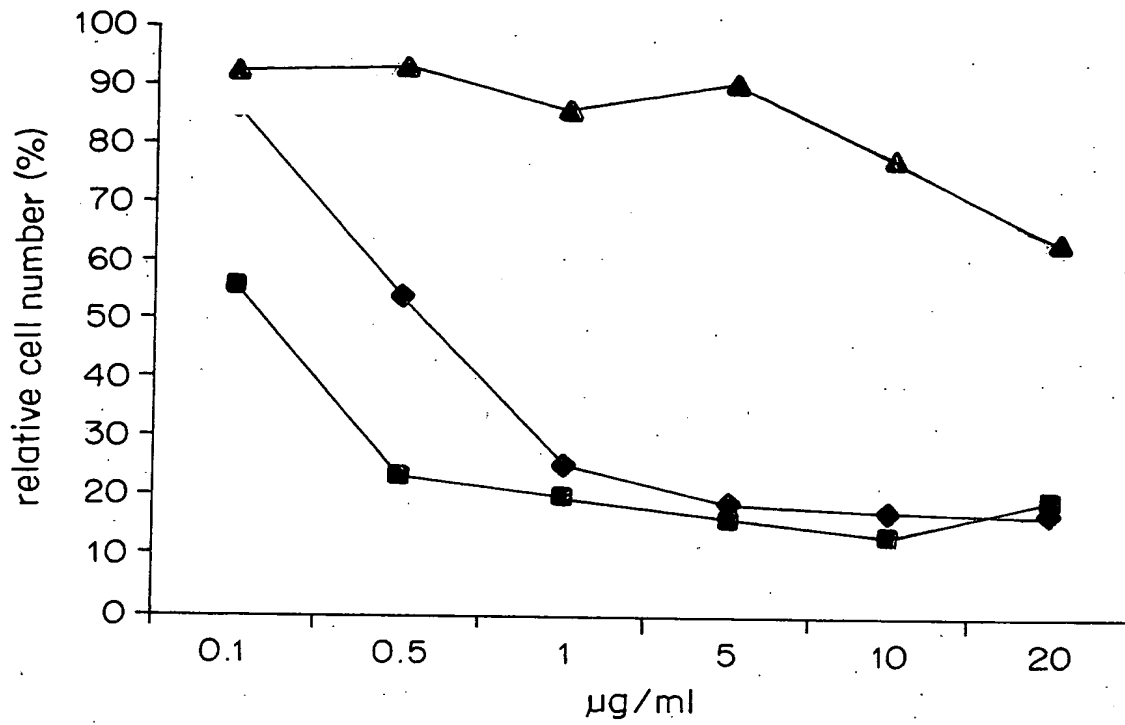


FIG. 40

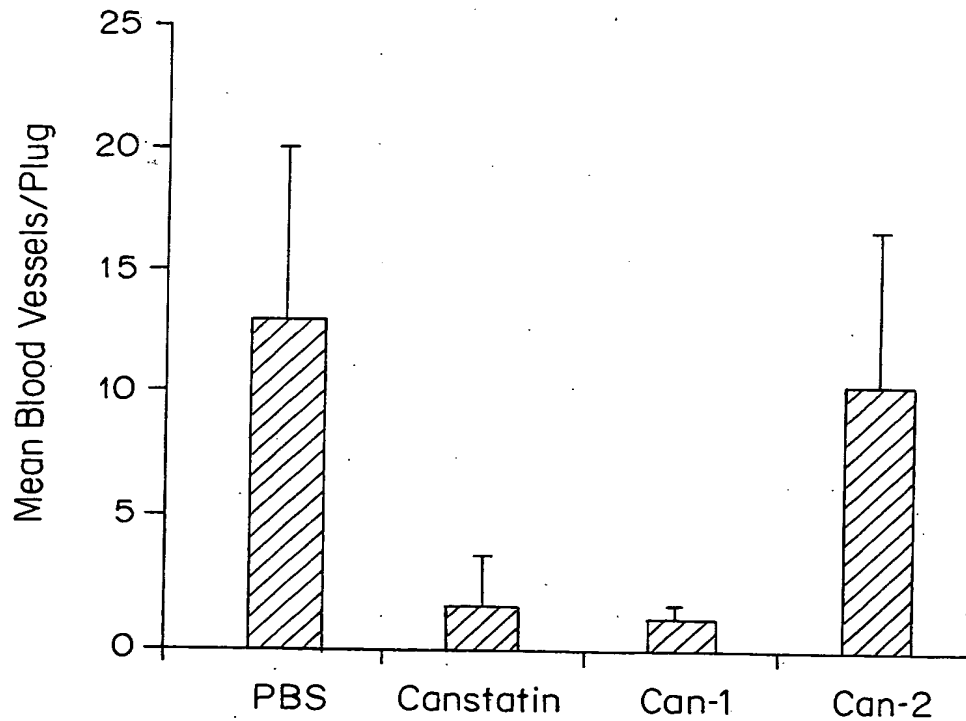


FIG. 41